

# The Journal of Parasitology

*The Official Organ of the American Society of Parasitologists*

Founded by HENRY BALDWIN WARD

VOLUME XXXI

1945

## EDITORIAL BOARD

**BENJAMIN G. CHITWOOD**  
*U. S. Bureau of Plant Industry*

**LOWELL T. COGGESHALL**  
*University of Michigan*

**WILLIAM W. CORT**  
*Johns Hopkins University*

**RUDOLF W. GLASER**  
*Rockefeller Institute*

**WILLIAM L. JELLISON**  
*U. S. Public Health Service*

**HAROLD KIRBY**  
*University of California*

**P. P. LEVINE**  
*Cornell University*

**JOHN T. LUCKER**  
*U. S. Department of Agriculture*

**CHARLES W. REES**  
*National Institute of Health*

**JUSTUS F. MUELLER**  
*Syracuse University*

**BENJAMIN SCHWARTZ**  
*U. S. Bureau of Animal Industry*

**LLOYD A. SPINDLER**  
*U. S. Department of Agriculture*

**NORMAN R. STOLL**  
*Rockefeller Institute*

## EDITORIAL COMMITTEE

**HORACE W. STUNKARD, Chairman**  
*New York University, University  
Heights, New York 53, N. Y.*

**WILLIAM A. RILEY**  
*University of Minnesota*

**DAVID H. WENRICH**  
*University of Pennsylvania*



THE SCIENCE PRESS PRINTING COMPANY  
LANCASTER, PENNSYLVANIA

# CONTENTS OF VOLUME 31

## FEBRUARY, 1945, NUMBER 1.

Bishopp, F. C. and Helen Louise Trembley. DISTRIBUTION AND HOSTS OF CERTAIN NORTH AMERICAN TICKS .....	1
Cantrell, William and Helen B. Jordan. NEW MOSQUITO HOSTS FOR <i>Plasmodium gallinaceum</i> .....	55
Van Cleave, Harley J. A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS <i>Illiosentis</i> (RHADINORHYNCHIDAE) .....	57
Cort, W. W., Sterling Brackett, Louis Olivier and L. O. Nolf. INFLUENCE OF LARVAL TREMATODE INFECTIONS IN SNAILS ON THEIR SECOND INTERMEDIATE HOST RELATIONS TO THE STRIGEID TREMATODE <i>Cotylurus flabelliformis</i> (FAUST, 1917) .....	61
Stabler, Robert M. INGESTION PROCESSES ON <i>Iodamoeba</i> (PROTOZOA) .....	79
RESEARCH NOTE.	
Hammond, Datus M. and David E. Bartlett. AN INSTANCE OF PHAGOCYTOSIS OF <i>Trichomonas foetus</i> IN BOVINE VAGINAL SECRETIONS .....	82

## APRIL, 1945, NUMBER 2.

Jellison, Wm. L. SIPHONAPTERA: THE GENUS <i>Oropsylla</i> IN NORTH AMERICA .....	83
Ripsom, C. A. and C. A. Herrick. EFFECTS OF VARIOUS SULFA COMPOUNDS ON THE PROTOZOAN PARASITE, <i>Eimeria tenella</i> .....	98
Cullinan, R. P. THE LARVAE OF <i>Eustrongylides ignotus</i> IN <i>Fundulus heteroclitus</i> .....	109
Hawkins, Phillip A. and C. L. Cole. STUDIES OF SHEEP PARASITES. V. IMMUNITY TO GASTROINTESTINAL NEMATODES .....	113
Addis, C. J. <i>Phlebotomus (Dampfomyia) anthophorus</i> N. SP., AND <i>Phlebotomus diabolicus</i> HALL FROM TEXAS (DIPTERA: PSYCHODIDAE) .....	119
Van Cleave, Harley J. A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS <i>Polymorphus</i> FROM THE AMERICAN COOT .....	128
Denton, J. Fred. STUDIES ON THE LIFE HISTORY OF <i>Brachylecithum americanum</i> N. SP., A LIVER FLUKE OF PASSERINE BIRDS .....	131
Rankin, John S., Jr. AN ECOLOGICAL STUDY OF THE HELMINTH PARASITES OF AMPHIBIANS AND REPTILES OF WESTERN MASSACHUSETTS AND VICINITY .....	142
RESEARCH NOTES.	
Schuck, Betty R. A NEW LOCALITY FOR <i>Trypanosoma cruzi</i> IN ARIZONA .....	151
Webster, J. Dan. INTESTINAL MYIASIS WITH <i>Lucilia</i> .....	151
Stunkard, Horace W. THE SYRIAN HAMSTER, <i>Cricetus auratus</i> , HOST OF <i>Hymenolepis nana</i> .....	151
AMERICAN SOCIETY OF PARASITOLOGISTS. THIRTY-FOURTH COUNCIL MEETING, BALTIMORE, MD., DECEMBER 16, 1944 .....	152

## JUNE, 1945, NUMBER 3.

Zuckerman, Lucile K. and Henry E. Meleney. A FLUID MEDIUM FOR THE ENCYSTATION OF <i>Endamoeba histolytica</i> UNDER REDUCED ATMOSPHERIC PRESSURE .....	155
Franks, Myron B. and Norman R. Stoll. THE ISOLATION OF MICROFILARIAE FROM BLOOD FOR USE AS ANTIGEN .....	158
Kirby, Harold. THE STRUCTURE OF THE COMMON INTESTINAL TRICHOMONAD OF MAN ...	163
Kirby, Harold. <i>Entamoeba coli</i> VERSUS <i>Endamoeba coli</i> .....	177
Fernando, Wilfred. THE STORAGE OF GLYCOGEN IN THE TEMNOCEPHALOIDEA .....	185
Matheson, Robert. DESCRIPTIONS OF TWO NEW SPECIES, <i>Paratrichobius andusei</i> AND <i>Nycteribosca franclemonti</i> (STREBLIDAE, DIPTERA, PUPIPARA) .....	191
Scott, Oliver K., Charles S. Richards and Elwood A. Seaman. EXPERIMENTAL INFECTION OF SOUTHERN CALIFORNIA MOSQUITOES WITH <i>Wuchereria bancrofti</i> .....	195
Reinhard, Edward G. <i>Paguritherium alatum</i> N. G., N. SP., AN ENTONISCAN PARASITE OF <i>Pagurus longicarpus</i> .....	198

- Leigh, W. Henry and Harley J. Van Cleave. METAMORPHOSIS OF THE FROG HOST AS A FACTOR IN CERCARIAL PENETRATION BY *Glythelminis quieta* ..... 205
- Self, J. Teague. A NEW TREMATODE, *Neorenilifer crotali*, FROM THE RATTLESNAKE ..... 210

## AUGUST, 1945, NUMBER 4.

- Jones, Arthur W. STUDIES IN CESTODE CYTOLOGY ..... 213
- Owen, William B. A NEW ANOPHELINE FROM THE SOLOMON ISLANDS WITH NOTES ON ITS BIOLOGY ..... 236
- Belkin, John N., Kenneth L. Knight and Lloyd E. Rozeboom. ANOPHELINE MOSQUITOES OF THE SOLOMON ISLANDS AND NEW HEBRIDES ..... 241
- Newton, Walter L. and Ivan Pratt. EXPERIMENTS TO DETERMINE WHETHER INFECTIVE LARVAE OF *Wuchereria bancrofti* CAN MIGRATE FROM THE ABDOMEN OF THE MOSQUITO INTERMEDIATE HOST ..... 266
- Hussey, Kathleen L. THE MIRACIDIUM OF *Proterometra macrostoma* (FAUST) HORSFALL, 1933 ..... 269
- Seitner, Philip G. STUDIES ON FIVE NEW SPECIES OF XIPHIIDOCERCARIAE OF THE VIRGULA TYPE ..... 272
- Wharton, G. W. *Trombicula frittisi* N. SP. (ACARINIDA: TROMBICULIDAE) ..... 282
- Chandler, Asa C. *Trichuris* SPECIES FROM CALIFORNIA RODENTS ..... 284
- Webster, J. Dan and C. J. Addis. HELMINTHS FROM THE BOB-WHITE QUAIL IN TEXAS ..... 286
- RESEARCH NOTES.
- Mauss, Evelyn A. PINWORM INFESTATION AMONG CHILDREN OF RURAL COMMUNITIES ..... 288
- Brackett, Sterling and Carrie Ola Hughes. CHILLING AS A MEANS OF RETAINING THE VIABILITY OF THE SPOROZOITES OF *Plasmodium gallinaceum* ..... 289
- Beltrán, Enrique. CONFLICTING VIEWS IN REGARD TO *Iodamoeba williamsi* ..... 290

## OCTOBER, 1945, NUMBER 5.

- Larsh, John E., Jr. EFFECTS OF ALCOHOL ON NATURAL RESISTANCE TO THE DWARF TAPEWORM IN MICE ..... 291
- Stunkard, Horace W. THE MORPHOLOGY OF *Tamerlania bragai* DOS SANTOS, 1934 ..... 301
- Maldonado, José F. THE LIFE CYCLE OF *Tamerlania bragai*, SANTOS 1934, (EUCOTYLIDAE), A KIDNEY FLUKE OF DOMESTIC PIGEONS ..... 306
- Belkin, John N. *Anopheles nataliae*, A NEW SPECIES FROM GUADALCANAL ..... 315
- Addis, C. J. LABORATORY REARING AND LIFE CYCLE OF *Phlebotomus (Dampfomyia) anthophorus* ADDIS (DIPTERA: PSYCHODIDAE) ..... 319
- Goble, Frans C. and H. L. Kutz. THE GENUS *Dispharynx* (NEMATODA: ACUARIIDAE) IN GALLIFORM AND PASSERIFORM BIRDS ..... 323
- Van Cleave, Harley J. THE ACANTHOCEPHALAN GENUS *Corynosoma*. I. THE SPECIES FOUND IN WATER BIRDS OF NORTH AMERICA ..... 332
- Griffiths, James T., Jr. A SCRUB TYPHUS (TSUTSUGAMUSHI) OUTBREAK IN DUTCH NEW GUINEA ..... 341
- AMERICAN SOCIETY OF PARASITOLOGISTS. PRELIMINARY ANNOUNCEMENT OF THE TWENTIETH ANNUAL MEETING ..... 351

## DECEMBER, 1945, NUMBER 6.

- Farr, Marion M. and Everett E. Wehr. SULFAMERAZINE THERAPY IN EXPERIMENTAL CECAL COCCIDIOSIS OF CHICKENS ..... 353
- Wehr, Everett E. and Marion M. Farr. EFFECT OF SULFAGUANIDINE ON THE COURSE OF INFECTION IN CHICKENS WITH *Eimeria tenella* ..... 359
- Hunter, George W. III and C. Brooke Worth. VARIATIONS IN RESPONSE TO FILARIFORM LARVAE OF *Ancylostoma caninum* IN THE SKIN OF MAN ..... 366
- Jellison, William L. A NEW MITE, *Laelaps aplodontiae*, FROM *Aplodontia* ..... 373
- Wenrich, D. H. THE CULTIVATION OF *Trichomonas augusta* (PROTOZOA) FROM FROGS ..... 375
- Brand, Theodor von. PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL *Eustrongylides*. VIII. INFLUENCE OF RESPIRATORY POISONS UPON THE AEROBIC GASEOUS METABOLISM ..... 381
- Goble, Frans C. and H. L. Kutz. NOTES ON THE GAPEWORMS (NEMATODA: SYNGAMIDAE) OF GALLIFORM AND PASSERIFORM BIRDS IN NEW YORK STATE ..... 394
- Wharton, G. W. TWO NEW SPECIES OF *Acariscus*: *A. pluvius* AND *A. anous* (ACARINIDA: TROMBICULIDAE) ..... 401

# CONTENTS OF VOLUME XXXI

iii

Reid, W. Malcolm. COMPARISON BETWEEN <i>in vitro</i> AND <i>in vivo</i> GLYCOGEN UTILIZATION IN THE FOWL NEMATODE <i>Ascaridia galli</i> .....	406
Manter, Harold W. <i>Dermadena lactophrysi</i> N. GEN., N. SP. (TREMATODA: LEPOCREADIIDAE) AND CONSIDERATION OF THE RELATED GENUS <i>Pseudocreadium</i> .....	411
Lynch, James E. REDESCRIPTION OF THE SPECIES OF <i>Gyrocotyle</i> FROM THE RATFISH, <i>Hydrolagus collieri</i> (LAY AND BENNET), WITH NOTES ON THE MORPHOLOGY AND TAXONOMY OF THE GENUS .....	418
BOOKS AND MONOGRAPHS RECEIVED .....	447
INDEX FOR VOLUME 31, NUMBERS 1-6 .....	449
INDEX FOR DECEMBER SUPPLEMENT .....	453



## DECEMBER SUPPLEMENT, 1945.

AMERICAN SOCIETY OF PARASITOLOGISTS.	
PROGRAM, 20TH ANNUAL MEETING, SAINT LOUIS, MISSOURI, MARCH 28-30, 1946 .....	1
AUTHOR INDEX .....	6
ABSTRACTS .....	7
OFFICERS .....	26
IN MEMORIAM .....	29
LIST OF NEW MEMBERS .....	30
APPLICATION FOR HOUSING ACCOMMODATIONS FOR ANNUAL MEETING .....	32





# The Journal of Parasitology

Volume 31

FEBRUARY, 1945

Number 1

## DISTRIBUTION AND HOSTS OF CERTAIN NORTH AMERICAN TICKS

F. C. BISHOPP<sup>1</sup> AND HELEN LOUISE TREMBLEY<sup>2</sup>

The importance of ticks as transmitters of a number of serious diseases and as parasites and annoyers of man and animals makes desirable the assembling of information on their geographical and seasonal distribution, and especially on their host relationships.

During the last 35 years representatives of the Bureau of Entomology and later the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, have collected specimens of ticks from many parts of the United States, and with the aid of correspondents have assembled numerous lots of these parasites with appropriate collection data.

The identifications have for the most part been made or verified by the senior author. A great many of the specimens collected in the earlier years of this period were determined by Nathan Banks, and some by W. A. Hooker and the late H. P. Wood. During this long period many individuals participated in the collection of specimens, in the accessioning and care of material, and in the rearing of some of the immature stages. This assistance is gratefully acknowledged, and the writers regret that it is not practicable to give specific credit to all those who have given assistance, but mention is made of the following whose contributions are especially noteworthy: W. A. Hooker, W. V. King, Harold S. Peters, George N. Wolcott, and Carroll N. Smith.

Although the formal data presented are based entirely on the collections of the Bureau of Entomology and Plant Quarantine, reference is made here and there to distribution and host records not represented in the Bureau files. Maps are used to indicate distribution of the more important and numerous species. The large dots in these maps represent specific points from which ticks in the Bureau collection were taken. Although distribution of these large dots is indicative of abundance, consideration must be given to the fact that more intensive collecting was carried out in some areas than in others. The small dots indicate the probable normal distribution of the species. This probable distribution takes into consideration records published by others than members of the Bureau staff.

Considerable information on the distribution and hosts of a number of North American ticks has been published by Hunter and Hooker (1907), Banks (1908),

Received for publication, October 25, 1944.

<sup>1</sup> Assistant Chief, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture.

<sup>2</sup> Assistant Entomologist, formerly with Bureau of Entomology and Plant Quarantine, at present with the Malaria Drug Testing Laboratory, National Institute of Health, Bethesda, Md.

Hooker (1908, 1909a, 1909b), Hunter and Bishopp (1911), Cooley (1911), Bishopp (1911a, 1911b, 1912), Birdseye (1912), Hooker, Bishop, and Wood (1912), Bishopp and King (1913), Bishopp and Wood (1913), Chamberlain (1937), and Cooley (1938). The data in the above publications that had been taken from the records of the Bureau of Entomology and Plant Quarantine are included in the present publication. Many others have published data in this field and some of these are referred to under the various species.

No effort has been made to list all known hosts or to present complete informa-

TABLE 1.—*Amblyomma americanum*: Host Records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
No host or unattached . . . .	56	1 (-100)*	20 (64)	36 (164)	38 (+123uf)
Badger . . . . .	1	.....	.....	1 (1)	1 (1u)
Bobcat ( <i>Lynx rufus</i> and <i>L. r. floridanus</i> ) . . . . .	2	.....	2 (7sp)	.....	1 (2s)
Cardinal . . . . .	1	.....	1 (1)	.....	.....
Cat (domestic) . . . . .	3	.....	3 (17)	.....	.....
Cattle . . . . .	124	9 (+36uf)	44 (+289uf)	58 (+225)	78 (+450uf)
Chaparral cock . . . . .	5	3 (77pf)	.....	.....	.....
Chicken . . . . .	1	.....	1 (1s)	.....	.....
Coyote . . . . .	1	.....	.....	1 (1)	1 (9)
Crow, southern . . . . .	1†	.....	.....	.....	.....
Deer, white-tailed . . . . .	20	15 (215uf)	44 (523uf)	35 (+402)	34 (129uf)
Dog . . . . .	106	13 (+52uf)	33 (145uf)	45 (+205)	74
Fox, gray and Florida gray . . . . .	11	6 (118uf)	6 (32up)	4 (6)	1 (1s)
Goat . . . . .	18	1 (2f)	7 (161sf)	8 (+12)	14 (65sf)
Hawk, sparrow . . . . .	1	1 (1f)	.....	.....	.....
Heron, little green . . . . .	1	.....	.....	1 (1)	.....
Hog . . . . .	30	1 (12)	15 (78uf)	22 (186)	22 (203up)
Horse . . . . .	26	1 (2)	8 (39uf)	14 (119)	22 (+146uf)
Idon, mountain . . . . .	1	.....	1 (1p)	1 (1)	1 (2s)
Man . . . . .	159	12 (+145u)	98 (+319up)	52 (+314)	67 (+32Suf)
Mink, Florida . . . . .	1	1 (1u)	.....	.....	.....
Mule . . . . .	7	.....	8 (26uf)	5 (+58)	6 (+80uf)
Opossum, Florida . . . . .	2	.....	2 (3us)	.....	.....
Oven bird . . . . .	1	1 (1f)	.....	.....	.....
Pocket-gopher, southern . . . . .	1	.....	1 (3sp)	.....	.....
Quail . . . . .	6	3 (59us)	5 (17up)	.....	.....
Rabbit . . . . .	1	1 (8u)	.....	.....	1 (2u)
Rabbit, Carolina marsh . . . . .	2	.....	2 (2us)	.....	.....
Rabbit, jack . . . . .	1	.....	1 (1f)	1 (1)	.....
Rabbit, swamp . . . . .	1	.....	1 (5sp)	.....	.....
Raccoon (several forms including <i>Procyon lotor chuscus</i> and <i>P. l. solutus</i> ) . . . . .	14	8 (220uf)	10 (313uf)	.....	1 (1)
Rat, eastern cotton . . . . .	1	1 (1)	.....	.....	.....
Rat, Norway . . . . .	1	1 (1f)	.....	.....	.....
Rat, rice . . . . .	1	.....	1 (1s)	.....	.....
Sheep . . . . .	10	.....	.....	5 (12)	10 (+47uf)
Skunk (several species including Florida skunk) . . . . .	5	1 (21)	4 (+17uf)	.....	.....
Squirrel (several species including fox, southern fox, western fox (introduced), red, rock, and gray) . . . . .	15	7 (70uf)	14 (+126uf)	.....	1 (1)
Squirrel, southern gray . . . . .	16	10 (37uf)	12 (31up)	.....	.....
Turkey, wild . . . . .	8	3 (22uf)	7 (49up)	.....	.....
Wolf (several species including red) . . . . .	4	1 (1p)	1 (3f)	.....	1 (5f)
Woodchuck, southern . . . . .	1	.....	1 (21pf)	.....	.....
Yellowthroat, Florida . . . . .	1	.....	1 (1u)	.....	.....

\* See text for significance of figures and symbols.

† 3 ticks the stage or stages of which were not recorded.

tion on the world distribution of the species treated. Some general idea of distribution outside the United States is given, however, for most species. In presenting the host data the number of the different hosts examined was not the same and other factors make direct comparisons difficult; however, the number of times a species was taken on a given host appears to give some slight indication of the relative importance of different animals as hosts for a tick species.

In the tables which give the hosts of the various ticks, the following method of indicating the collection data has been adopted to permit condensing of the informa-

tion. The number of separate lots is given in the first column following the name of the host. In the columns that follow, the first number gives the number of lots that included the stage at the head of the column, in parentheses is given the number of individuals, usually with an indication of the state of engorgement, in which case "u" signifies unengorged, "s" slightly engorged, "p" partly engorged, and "f" fully engorged. Thus in Table 1, in the data for man there were in the collections 159 lots, 12 lots included more than 145 unengorged larvae, 98 lots included more than 319 unengorged to partly engorged nymphs, 82 lots included more than 314 males, and 67 lots more than 328 unengorged to fully engorged females. Data that could not be exactly classified are given in footnotes. Males of ixodid ticks do not become distended, and so no degree of engorgement is indicated for species of that group.

Hosts are referred to by common names, and a list of scientific names is given at the conclusion of the article.

### *Amblyomma americanum* (L.)

#### The Lone Star Tick

The lone star tick is among the more economically important species in the United States. As a parasite it is important because of the fact that it attaches to man in all its active stages, and because its long mouth parts are deeply embedded in the host and often broken off when the tick is removed, thus producing persistent and severe irritation. Its great abundance on various wild and domestic hosts causes much annoyance and loss of blood. This species is also a predisposing cause of screwworm attack. As a disease carrier it assumes importance since it is capable of transmitting tularemia and Rocky Mountain spotted fever. In the South this species probably plays a larger part in this respect than is recognized. Recently the occurrence in nature has been reported of nymphs infected with the causative organism of American Q fever (*Rickettsia diaphorica*) in Liberty County, Texas.

#### Distribution

This tick is abundant in the States bordering the Gulf of Mexico. It is plentiful along the South Atlantic coast and in parts of Oklahoma, Arkansas, and Missouri. Occasional specimens are collected in States to the North. There is much local variation in the abundance of this tick. It is to be found in especially great numbers in wooded areas, particularly where underbrush is dense, such as along river-bottoms in prairie areas, and in the canebrakes of Louisiana and Mississippi. This tick has become notoriously abundant in the Ozark region in recent years, and the collection of specimens on several different dates at Osceola, Iowa, by one of our collaborators, G. S. Cantonwine, indicates that the species is well established there.

Its present occurrence in the more northern States such as Connecticut and Michigan is probably accidental, resulting from introductions on people or domestic animals. The single record (1 unfed female on dog) from Chicago, Ill., might be attributable to the livestock trucks which the dog's owner says pass the house. An unfed female from Labrador is in the Marx collection. The species has been recorded from Guatemala, "Guiana," and Brazil and undoubtedly occurs in the Gulf Coast region of Mexico.



Fitch (1872) stated that although the lone star tick was formerly common in New York State, he had seen only a single specimen from there—one collected in 1830. There is reason to believe that this species was more numerous in the Northern States many years ago. Cooley and Kohls (1944a) have presented a number of detailed records of collections. These do not extend the distributions beyond that indicated by our collections as shown on the map in Fig. 1.

The type locality is Pennsylvania or New Jersey.

#### Hosts

The lone star tick is a three-host species, and is a general feeder in all its active stages. Our records indicate that certain ground-inhabiting birds, such as quail and wild turkey, are commonly infested with larvae and nymphs, yet there is no evidence of domestic turkeys, chickens, or other poultry being heavily infested. This

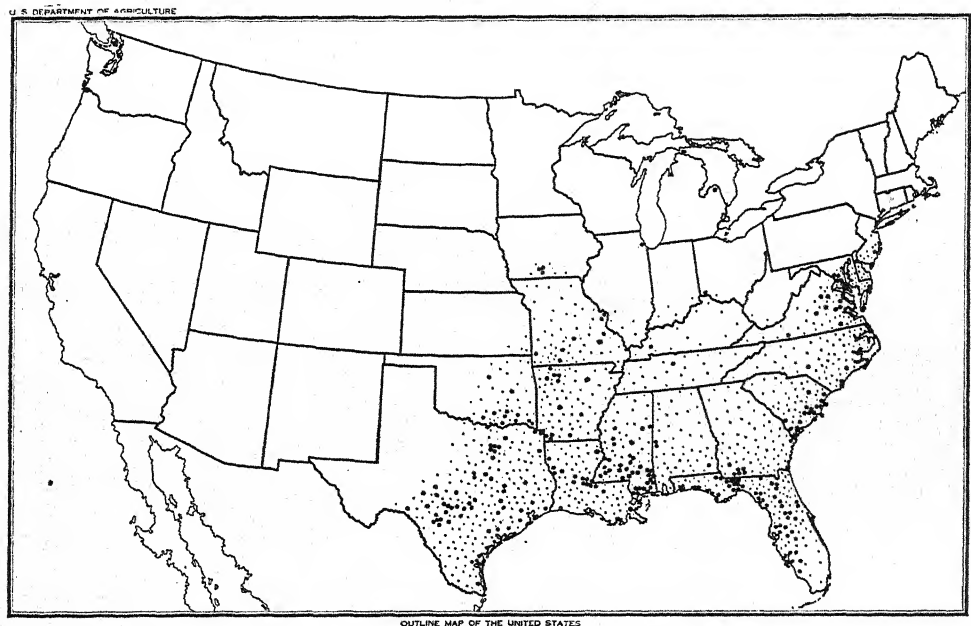


FIG. 1. Distribution of *Amblyomma americanum* in the United States. Large dots indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

is probably due mainly to lack of exposure of these hosts in heavily infested areas. It is interesting to note that wild turkeys on Bull's Island, S. C., have been observed by Messrs. Blakey and Baldwin to be very heavily infested with larvae and nymphs. In fact, there is some reason to attribute mortality of wild turkeys to gross infestations of this species. Rabbits, for some reason, appear rarely to be infested. Deer are often very heavily infested with all stages. The authors have observed specimens of Virginia deer that were literally covered with larvae, nymphs, and adults in various stages of engorgement. The ears, both inside and outside, are favorite places of attachment.

A careful estimate was made of the number of ticks on 1 ear of a deer at Mt. Pleasant, S. C., in September 1937. There were approximately 4,800 specimens, most of which were nymphs of *Amblyomma americanum*, although a few males

of *A. americanum* and a few females of *A. maculatum* were present. The other ear was also heavily infested. The ears under such circumstances become thickened, scabby, and partly denuded of hair. The ticks also attach to and engorge on the antlers when these are in the velvet.

In the Southern States this tick has been taken in all stages on hosts throughout the year, although it is less abundant during midwinter.

Host records of the Bureau of Entomology and Plant Quarantine are given in Table 1.

*Amblyomma cajennense* (F.)

The Cayenne Tick

The cayenne tick is a native of North, Central, and South America. The first collection and type material of this species came from Cayenne, French Guiana. It is a species of much economic importance in the tropics of the Americas, where it is generally abundant and active the year round. It has been shown to be a carrier of Rocky Mountain spotted fever in Brazil, and it is a very troublesome pest of man and of all classes of livestock.

*Distribution*

Distribution of this tick in the United States appears to be confined to extreme southern Texas (Fig. 2). The Marx collection contains an unengorged female

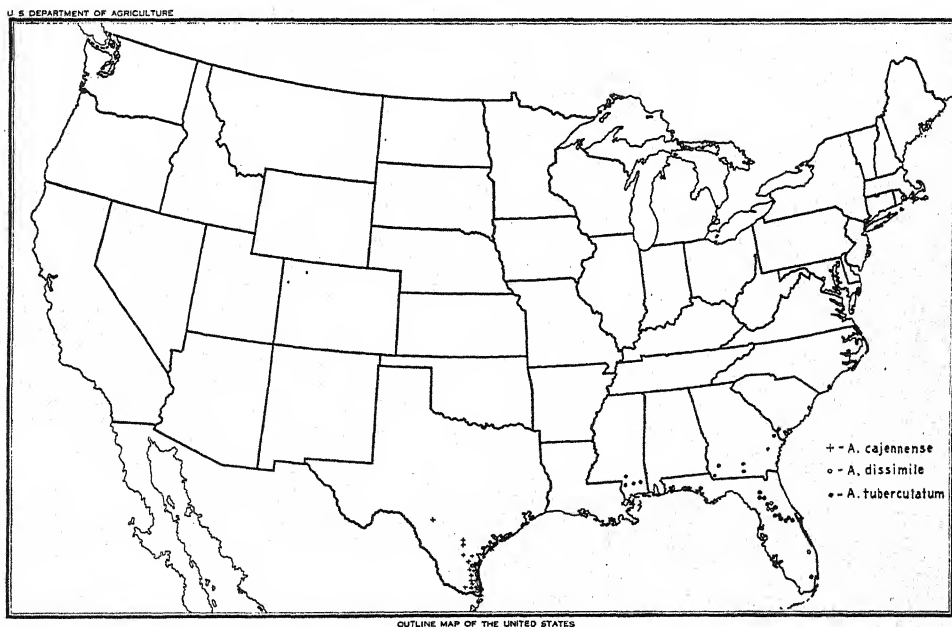


FIG. 2. Distribution of *Amblyomma cajennense*, *A. dissimile*, and *A. tuberculatum* in the United States, as indicated by collections of the Bureau of Entomology and Plant Quarantine.

labeled "Biscayne Bay, Fla."; however, this species does not appear to be established in that State. As pointed out by Hooker, Bishopp, and Wood (1912), records of the occurrence of this tick in Arizona are probably based on a jar of ticks incorrectly labeled. A collection from San Diego County, Calif., is recorded by Banks (1908). The collection of the Bureau of Entomology and Plant Quarantine includes speci-

mens from the following counties in Texas: Aransas, Cameron, Hidalgo, Jim Wells, Kennedy, Kleberg, Liveoak, Nueces, Uvalde, and Willacy. It also contains specimens from Mexico, Cuba, Jamaica, Guatemala, Honduras, Panama, Colombia, Venezuela, and Brazil. Other countries from which this tick has been recorded are the following: Nicaragua, Costa Rica, Bermuda, Trinidad, French Guiana, Paraguay, and Argentina. Cooley and Kohls (1944a) record specimens from two additional southern Texas counties, Starr and Brooks.

### Hosts

All stages of this species attach to man as well as to livestock; and the long mouth parts make removal difficult, often breaking off to remain in the flesh and cause painful wounds. In many parts of tropical America the larvae and nymphs are extremely abundant and aggressive in attacking man. All stages are active and are found on hosts throughout the year. Their small size and activity make their exclusion from clothing very difficult.

Bureau of Entomology and Plant Quarantine records from the United States are given in Table 2.

TABLE 2.—*Amblyomma cajennense*, *A. dissimile*, and *A. tuberculatum*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
<i>Amblyomma cajennense</i>					
Ass .....	2	....	2 (2uf)	....	....
Cattle .....	5	....	2 (28uf)	1 (1)	4 (4up)
Coyote .....	3	1 (3p)	3 (9sp)	2 (5)	1 (2us)
Dog .....	3	....	....	1 (2)	3 (5up)
Horse .....	9	1 (1sp)	2 (23f)	6 (9)	8 (9uf)
Lion, Mexican .....	1	....	....	....	1 (1s)
Man .....	6	1 (1p)	3 (10uf)	1 (1)	3 (3u)
Peccary, Texas .....	7	....	5 (19sp)	5 (13)	4 (7uf)
Raccoon .....	1	....	1 (3up)	....	....
Turkey, wild .....	1	....	1 (3p)	....	....
<i>Amblyomma dissimile</i>					
Boa, Mexican .....	2	....	1 (1s)	1 (1)	1 (1s)
Ctenosaura, spiny .....	1	....	....	1 (1)	....
Iguana .....	6	....	3 (27p)	6 (41)	3 (23uf)
Lizard ( <i>Scalopus undulatus</i> ) .....	1	1 (15pf)	....	....	....
Rattlesnake, diamond .....	2	....	1 (5pf)	....	1 (1f)
<i>Amblyomma tuberculatum</i>					
Cattle .....	1	1 (3up)	....	....	....
Chicken .....	1	1 (10uf)	....	....	....
Dog .....	7	7 (+ 89uf)	....	....	....
Hawk .....	1	1 (6uf)	....	....	....
Meadowlark, southern .....	1*	....	....	....	....
Rabbit, cottontail .....	1	1 (75)	....	....	....
Squirrel, fox .....	1	1 (75)	....	....	....
Terrapin, diamond-back .....	1	....	....	....	1 (1)
Thrush, hermit .....	1	1 (1f)	....	....	....
Tortoise, gopher .....	28	....	13 (65up)	21 (52)	13 (27uf)

\* Recorded as "tick." No stage indicated.

Horses and mules in the vicinity of Tampico, Mexico, were noted by the senior author to have numerous males and females of this species attached to them.

Foreign records include the following hosts: Game cock, burro, cattle, deer, dog, horse, and man.

### *Amblyomma dissimile* Koch

#### The Iguana Tick

The iguana tick is of no known economic importance, although in some instances it may be injurious to reptiles in captivity.

### Distribution

The iguana tick is a native of tropical America, ranging from Mexico and the West Indies to Argentina. The type locality is Mexico. Our collections do not contain any specimens taken in nature in the United States, but specimens are not infrequently seen on reptiles and amphibians in zoological gardens, especially in localities where such animals are first brought in from the Tropics (Fig. 2).

Bequaert (1932) reports a collection from a snake in nature at Boca Raton, Palm Beach County, Fla., and Sebastian, Indian River County, Fla.

Outside the United States, the species is recorded from Mexico, Guatemala, British Honduras, Republic of Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Peru, British Guiana, Brazil, Paraguay, Argentina, Tobago, Grenada, Saint Lucia, Antigua, Barbados, and Jamaica.

### Hosts

This species attaches exclusively to cold-blooded animals (Reptilia and Batrachia) in nature, although, as reported by Hooker, Bishopp, and Wood (1912), it has been engorged in the larval and nymphal stages on bovines. These authors were unable to get the adults to attach to warm-blooded animals. Robinson (1926) also lists adults as having been taken from cow (1 lot) and sheep (1 lot), and Neumann (1911) lists capybara as a host. Newstead (1909) states that in Jamaica it is apparently confined to the toad *Bufo marinus* and that on this host it often occurs singly, but that occasionally four or five specimens of various stages were found on a single host. Bequaert's (1932) specimens were from gopher snake (*Spilotes corais cooperi*) and pigmy, or ground rattlesnake (*Sistrurus miliaris*).

The records of hosts of the iguana tick (all in captivity) in the Bureau collections from the United States are shown in Table 2.

### *Amblyomma maculatum* Koch

#### The Gulf Coast Tick

The Gulf Coast tick is a rather important pest of livestock in the States bordering the Gulf of Mexico. The adults attach in the external ears and when numerous produce intense inflammation and swelling. This often induces infestations of the screwworm, which may result in the death of the animal. It may also cause the destruction of the supporting cartilage of the ears, especially in the case of horses and mules. This permits the ears to droop over, a condition popularly known as "gotch ear."

### Distribution

This tick is a native of North, Central, and South America. In the United States its distribution is restricted to the Gulf Coast and Coastal South Atlantic regions from South Carolina to Texas, inclusive (Fig. 3). It is most abundant in the area within 100 miles of the coast. A few specimens have been collected at interior points, such as at Dallas, Tex., Memphis, Tenn., and East Saint Louis, Ill. Those taken in the last two localities were probably all shipped in on livestock as at least some of them were on cattle that had been received recently from coastal areas. However, we received from Chapman and Bernard, Tulsa, Okla., a collection of 7 males and 7 females of the Gulf Coast tick taken from horses and cattle



on their ranch near Tulsa on June 22, 1944. They stated that this is the first time this tick has been noticed in the locality and yet it is very prevalent this year and getting into the ears of cattle and horses. No livestock had been shipped into the locality from the Gulf Coast area. They comment that there has been an unusual amount of rain in March and April, which may have something to do with the appearance of this pest. When the duration of attachment of the males and the fact that the larvae and nymphs often engorge on migratory birds is considered, it is surprising that more ticks of this species have not been found north of the normal range.

Niles (1898) collected ticks in Virginia in 1898 which were referred to as *Dermacentor occidentalis*, but Prof. H. A. Morgan has pointed out that these were

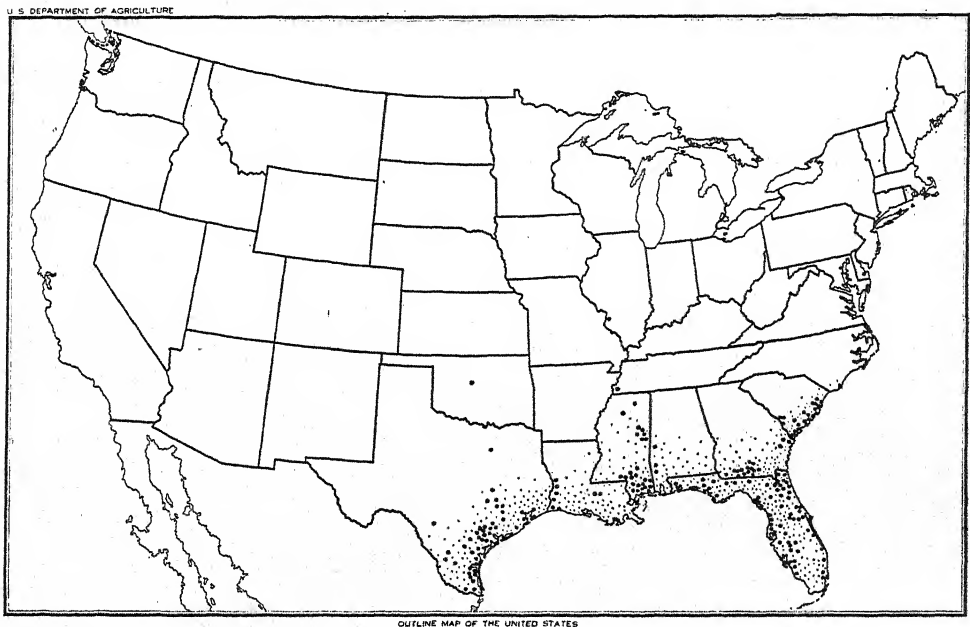


FIG. 3. Distribution of *Amblyomma maculatum* in the United States. Large dots indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

*Amblyomma maculatum*. The Marx collection contains a male specimen labeled by Marx "Tulare Co., Calif." This was probably an introduction on a host, as was probably the Virginia specimen. Cooley and Kohls (1944a) record two collections, similarly out of range, from Willcox, Ariz., and Weathers, Okla. Certainly this species is not common north of South Carolina.

The tick is known to occur abundantly along the Gulf Coast in Mexico and has been collected in Jamaica, Colombia, Brazil, Paraguay, Uruguay, Patagonia, Ecuador, Peru, and Chile.

The type locality is "Carolina."

#### Hosts

The Gulf Coast tick is a 3-host species. The larvae and nymphs engorge mainly on ground-inhabiting birds, although they are found not infrequently on small mammals. On birds they attach chiefly around the head. As many as 289 larvae have been taken on a single meadowlark (Bishopp and Hixson, 1936). On

October 26, 1937, a total of 152 ticks were taken by H. M. Brundrett from a meadowlark at Penny Farms, Fla. Of these, 98 were larvae of *Amblyomma maculatum* and others were *Haemaphysalis chordeilis* and *H. leporis-palustris*. Sheep, mules, horses, and cattle, in about the order named, suffer most from attack of the adults.

The adults are seldom found on hosts in the United States during the late winter and spring. They increase rapidly after the middle of July and become most abundant in late summer and early fall. Immature stages are found on birds throughout the year, but are less abundant in winter.

TABLE 3.—*Amblyomma maculatum*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Cloth dragged over vegetation .	15	2 (3u)	....	8	11(+ uf)
Bear	1	....	....	1(1)	....
Blackbird, Brewer's	1	....	1(1f)	....	....
Blackbird, redwinged	2	....	2(3pf)	....	....
Bluebird	1	....	1(1s)	....	....
Bobcat	1	....	....	1(1)	....
Cardinal	2	1(1)	1(8)	....	....
Cat, domestic	2*	....	....	1(1)	....
Cattle	75	....	....	53(+ 271)	55(+ 131uf)
Cowbird	1	1(i)	1(10sp)	....	....
Coyote	1	....	....	....	1(1s)
Deer, white-tailed	19	....	....	15(55)	8(+ 9)
Dog	88	....	....	31(+ 59)	22(+ 52uf)
Fox, gray	2	....	....	1(2)	1(3pf)
Goat	3	....	1(1)	2(13)	2(17up)
Grackle	2	....	2(3pf)	....	....
Hog	12	....	....	8(13)	11(22uf)
Horse	18	....	....	12(+ 25)	14(+ 42uf)
Killdeer	1	....	1(1s)	....	....
Man	4*	....	....	1(1)	2(6u)
Meadowlark	72	19(+ 170uf)	65(+ 123uf)	....	....
Mockingbird	2	2(8sp)	....	....	....
Quail	4	1(30sf)	3(9sf)	....	....
Rabbit, cottontail	2	....	2(7sf)	....	....
Rabbit, jack	1	....	1(6s)	....	....
Raccoon	1	....	1(1u)	....	....
Rat, cotton	1	....	1(1f)	....	....
Rat, rice	1	1(50f)	....	....	....
Sheep	23	....	....	13(+ 42)	19(+ 119uf)
Skunk	1	....	....	....	1(1u)
Sparrow, Bachman's	1	....	1(2sp)	....	....
Sparrow, Florida grasshopper	2	....	2(6f)	....	....
Sparrow, swamp	1	....	1(1p)	....	....
Squirrel, fox	2	....	2(4)	....	....
Thrush, wood	1	....	1(1f)	....	....
Towhee (several species, including red-eyed and white-eyed)	5	4(+ 11pf)	2(+ 1s)	....	....
Warbler, palm	1	1(9f)	....	....	....
Wolf	1	....	....	1(2)	1(1u)
Woodpecker, red-bellied	1	....	1(1s)	....	....
Wren, Carolina	2	2(+)	....	....	....
Wren, short-billed marsh	2	....	2(2p)	....	....

\* 1 lot labeled "ticks."

Bishopp and Hixson (1936) record collections in Georgia of larvae from the following hosts not listed above: bluejay, *Cyanocitta cristata cristata*; mockingbird, *Mimus polyglottos*; migrant shrike, *Lanius ludovicianus*; gray squirrel, *Sciurus niger niger*; cotton rat, *Sigmodon hispidus hispidus*; and sheep. A nymph was also recorded by them from the Carolina wren. These authors record the rearing of larvae, under laboratory conditions, on the following hosts: Junco (*Junco hyemalis*), guinea pig, meadowlark, pine mouse (*Pitymus pinetorum pinetorum*), white-footed mouse (*Peromyscus maniculatus*), quail, or bobwhite, cotton rat, roof rat, English sparrow, woodpecker (*Centurus carolinus*), and pigeon.

Hosts of this tick recorded from Mexico, the West Indies, and South America include the following: *Corvus campestris*, *Podinoma teguixin*, *Canis azarae*, and *C.*

*griseus*. Nymphs are reported by Aragão (1911) from *Nothura maculosa* (?) and *Rhynchotus rufescens*.

Host records of the Bureau of Entomology and Plant Quarantine are given in Table 3.

*Amblyomma tuberculatum* Marx

The Gopher-Tortoise Tick

The gopher-tortoise tick is of little economic importance because its principal host is the land tortoise, *Gopherus polyphemus*, so abundant in Florida. With perhaps one exception, *Amblyomma varium*, this is the largest species of tick known. The engorged female attains a length of 18 to 24 mm., and the engorged nymphs, 8 to 10 mm. In a few instances the larvae have been found to be annoying to chickens as a result of attachment in considerable numbers.

*Distribution*

The gopher-tortoise tick has been reported only from Florida and Alabama by Hooker, Bishopp, and Wood (1912), and from Cuba by Neumann (1899). It is rather abundant in central Florida, but much less so in western Florida and the southern parts of Alabama, Georgia, Mississippi, and South Carolina. Its distribution is approximately co-extensive with that of the gopher-tortoise (Fig. 2).

The species is apparently rare in the West Indies. Tate (1941) has examined many specimens of cold-blooded animals in Puerto Rico without finding it. The record from Cuba by Neumann referred to a single male (Gundlach coll. Paris Mus.).

*Hosts*

Adults of the gopher-tortoise tick confine their attack to the common land tortoise of the Southeastern States and the West Indies. They attach for the most part to the softer parts of the animal, as around the legs, tail, and neck, but not infrequently are found on the edge of the shell. This is particularly true of the males, which remain on the host long after the females become engorged and drop. It is probable that the adults never feed on warm-blooded animals. They have been engorged on box tortoise (probably *Terrapene ornata*) according to Hooker, Bishopp, and Wood (1912, p. 125).

The land tortoise is the principal host of the nymphs. In fact, our collection records fail to reveal their presence on any other animal in nature. However, they have been engorged on bovines under laboratory conditions.

The larvae feed on warm-blooded animals and birds, and they may occur on chickens, as previously stated, in considerable numbers. They have never been taken on cold-blooded animals by the writers.

The adults have been taken on the host throughout the year, and the records on larval collections indicate that they are probably present on hosts the year round, although our collections were made mainly during the cooler months, especially in December.

A list of the host records based on material examined by us is given in Table 2.

*Argas miniatus* Koch

The Fowl Tick

The fowl tick is a poultry pest of first importance in the Southwestern States, and in many places in warm-temperate, subtropical, and tropical countries of the

globe. This tick not only transmits fowl spirochaetosis, but it causes a form of paralysis among fowls and is a source of heavy loss to poultry raisers through the irritation and loss of blood due to its feeding on fowls of all ages. It is usually associated with domestic fowls, but it may be abundant in the roosting places of colonies of such wild birds as turkeys and vultures.

#### Distribution

As indicated above, this tick is widely distributed in the warm-temperate and tropical parts of the world. It is much less important as a pest in the more humid regions than in the arid sections, and its northern distribution in the United States is very definitely limited by low temperatures (Fig. 4). Although specimens have

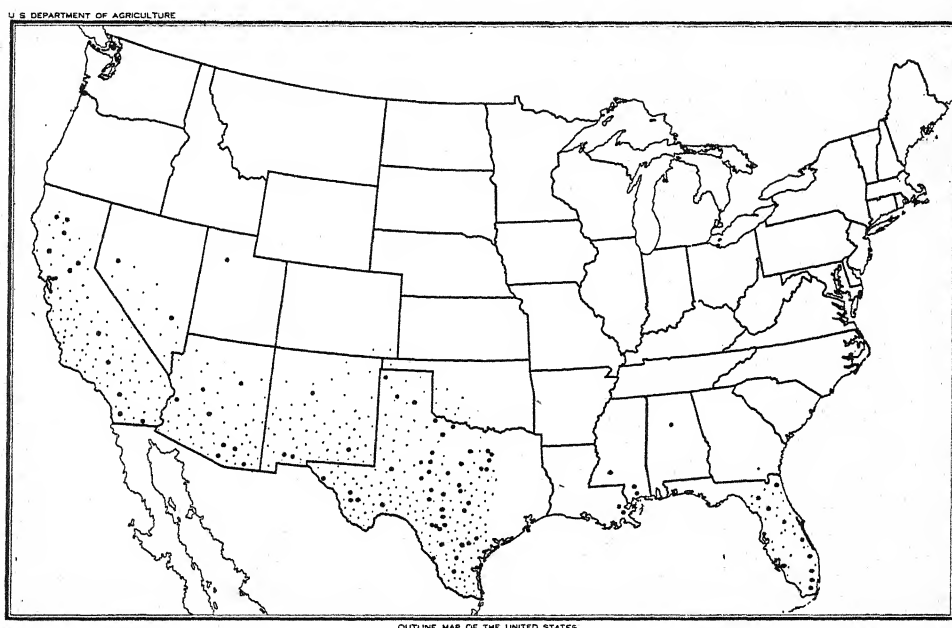


FIG. 4. Distribution of *Argas miniatus* in the United States. Large dots indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

been collected as far north as Iowa, and Portland, Ore. It appears that extreme northern records were based on introductions which did not persist. Hearle (1938) reported the identification of four nymphs collected (probably as larvae) on the golden-crowned sparrow at Vancouver, B. C., on May 2, 1931. He concluded that the bird had probably picked up the infestation in a chicken yard in California. During the last 40 years this tick has spread considerably through the Southern States, although it has not become generally distributed in the regions of heavy rainfall except in Florida. Dr. Clay Lyle has written us that the fowl tick does not occur in Mississippi except where it is introduced on poultry grown in the West. He states, "In addition to Biloxi it has been found in Wiggins, Brookhaven, and Meridian. The Wiggins infestation was reported June 22, 1935, and the Brookhaven infestation October 13, 1943. In all cases efforts have been made to eradicate the infestations as soon as possible."



## Hosts

Domestic fowls are by far the most important hosts of *Argas miniatus*, and chickens and turkeys are more frequently and more heavily infested than ducks or geese. Apparently the habits as well as the nature and density of the feathers tend to give ducks, geese, and pigeons much freedom from attack. Wild birds found infested were usually rather closely associated with poultry. Vultures may be somewhat of an exception to this, although vultures frequent barnyards and slaughter pens and may easily pick up infestations of seed ticks in such places and carry them to their roosts to become established. In two instances in which vulture roosts were investigated, the trees in which large numbers of the birds roosted were all infested with the tick in various stages of development. Under the bark and in crevices of one tree literally thousands of the ticks were present.

Reports of the fowl tick annoying man and livestock have been received, but it is evident that mammals are not preferred hosts. In most occurrences of this kind, poultry were, or had been, kept in close association with man or the animals annoyed. In one case in California a family that had made its home temporarily in a chicken

TABLE 4.—*Argas miniatus*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Dog*	1	1 (13pf)	1 (1)	....	1 (1)
Dove	1	1 (8p)	....	....	....
Duck, domestic	1	1 (1p)	....	....	....
Hawk, Swainson's	1	....	†	†	†
Man	2	1 (12sf)	....	....	....
Quail	1	1 (6sp)	....	....	....
Quail, California valley	1	1 (2f)	....	1 (2)	1 (2)
Thrush	2	1 (21uf)	....	....	....
Turkey, domestic	2	2 (8up)	Many	Many	(+ 150uf)
Turkey, wild	3	1 (1s)	....	....	....
Vulture (turkey and black)	3	....	....	....	....

\* Ticks were submitted by a correspondent, who said they were from a dog.  
† Number and stage unknown.

house reported that the ticks were biting them and causing much annoyance. One nymph was sent in from Calexico, Calif., with the report that it was biting man in bed and produced "an angry swelling with a white center."

The writers have a few other records of this tick attacking man, but these were instances in which poultry was closely associated with human habitations. Furthermore, the extent to which they actually insert their hypostomes into man, or draw blood, in the cases mentioned above, or others, has not been clearly established. The senior author, in working with this tick in all stages for a number of years, has never observed any tendency for it to attack man. This tick is called the "Miana bug" in Persia, and it is purported to be a pest there of man; however, it is likely that *Ornithodoros*, which bites man freely, may be confused often with the fowl tick.

Statements have been made by farmers that this tick annoys horses stabled in buildings in which chickens have roosted. Whether the ticks bite the horses or annoy only by crawling on them has not been established. Hooker, Bishopp, and Wood (1912) record the J. D. Mitchell collection of 3 adults on a jack rabbit in Texas. There appears to be a possibility that two lots of specimens may have been confused when this collection was made. These authors failed to get larvae confined on a bovine to attach, and were unable to get them to engorge on pigeons.

They partially engorged a single adult on a guinea pig. Nuttall and Warburton (1908, p. 84) obtained engorgement on rats and mice, but with difficulty.

Of 170 collections recorded in the Bureau accession files, 153 are from chickens or poultry houses. Other collections are shown in Table 4.

#### *Dermacentor albipictus* Pack.

##### The Winter Tick

The winter tick is a pest of importance on horses, cattle, and certain wild animals, especially moose and elk. Horses, particularly colts, on the range are often so heavily infested as to be weakened or even killed by it. Moose and elk die from gross infestation combined with feed shortages in late winter and early spring. There is some evidence that this tick is capable of transmitting Rocky Mountain spotted fever, although it can be of little importance in this respect because of its restricted host relations.

This is a one-host species, the larval and nymphal molts being passed on the host. Its seasonal activity is confined to the late fall, winter, and spring, when infestations are easily overlooked in the long winter coats of animals. When feed is short, the injurious effects are intensified.

##### Distribution

The winter tick is widely distributed in the Northern and Western States and in Canada (Fig. 5). It undoubtedly occurs in northern Mexico. The species is very unevenly distributed within its normal range. It appears to reach maximum abundance in the extreme northern States from Maine to Washington and Oregon. It is considered by Hearle (1938) to be one of the commonest and most widespread of Canadian species.

If *Dermacentor nigrolineatus* is regarded as a synonym of *D. albipictus* or as a variety of that species the distribution is extended to all parts of the United States. The senior author has been inclined to this view since the collection of forms in western Texas in 1921 that were intermediate between the extremes described by Packard. Cooley (1938) regards the species as synonymous. In this article, however, we are treating the forms as distinct species.

The tick remains on a host for 1 to 2 months, which gives abundant opportunity for its shipment from one part of the country to another. For instance, there are records of its shipment on elk from northern Wyoming to northern Colorado, and on a horse from Capitan, N. Mex., to Pocohontas, Miss.

##### Hosts

This tick is commonly found on the larger domestic and wild animals. It appears to prefer horse, elk, and moose, but is often abundant on cattle and deer. The seasonal occurrence on hosts varies somewhat with latitude and altitude. It is seldom found on hosts in the fall earlier than the end of September or in spring later than early June. For instance, adults have been collected in Los Angeles County, Calif., October 6, 1932, and in Badlands, N. Dak., on June 3.

Collection records of the Bureau of Entomology and Plant Quarantine are given in Table 5.

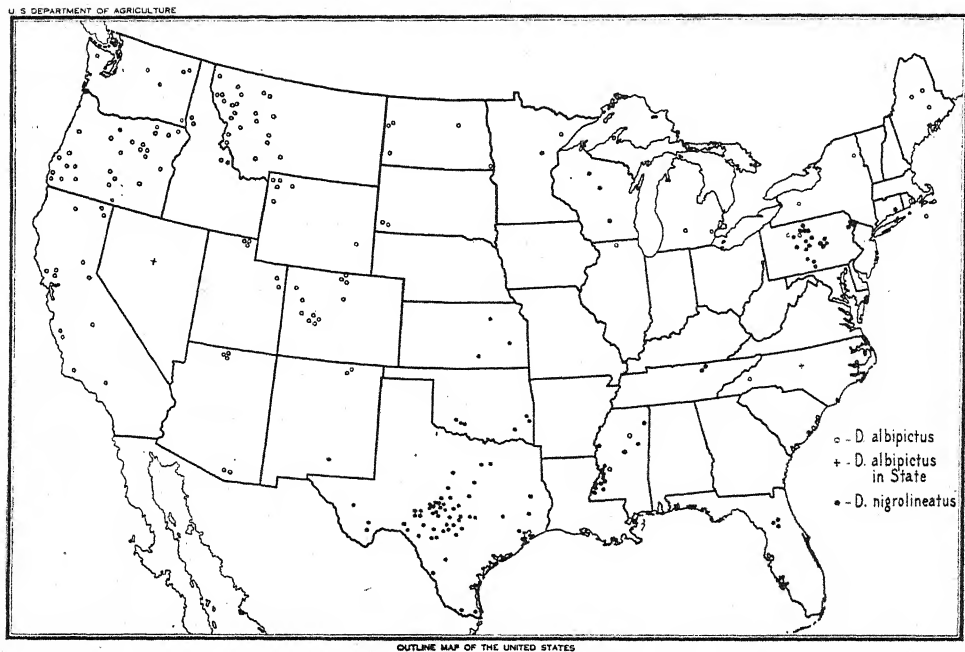


FIG. 5. Distribution of *Dermacentor albipictus* and *D. nigrolineatus* in the United States.

*Dermacentor andersoni* (Stiles)

Rocky Mountain Spotted Fever Tick

*Dermacentor andersoni*, known to many as *D. venustus* Banks, is of special interest because of its role in the transmission of Rocky Mountain spotted fever, Colorado tick fever, tularemia, and American Q fever, in the production of tick paralysis, and in the annoyance of man and other animals by its attack. Also Syverton and Berry (1936) have reported the experimental transmission of encephalomyelitis by it. The reputation of this tick as a disease carrier has an adverse influence on the development and utilization for recreation and other purposes of certain areas where it is abundant. Although this species has been intensively studied by members of the staff of the U. S. Public Health Service, the State Board of Ento-

TABLE 5.—*Dermacentor albipictus*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Antelope .....	2	....	1(2uf)	2(12)	2(10uf)
Caribou .....	1	....	1(1)	1(1)	1(1u)
Cat* .....	1	....	1(3f)	1(3)	1(1u)
Cattle .....	11	....	5(23pf)	7(20)	9(+26uf)
Deer .....	14	1(1f)	9(+44pf)	8(+100)	12(198uf)
Deer, black-tailed ( <i>Odocoileus hemionus</i> ) .....	26	....	11(+38uf)	15(+27)	19(+78uf)
Deer, Virginia .....	2	....	1(2f)	2(0)	2(10sp)
Elk .....	23	....	6(15uf)	12(+63)	21(+214uf)
Elk, western wapiti .....	8	1(2u)	6(42uf)	5(17)	5(21sf)
Goat, mountain .....	1	....	1(2)	1(many)	1(many)
Horse .....	137	1(2s)	46(+391uf)	94(+515)	123(+912uf)
Man .....	1	....	....	1(1)	....
Moose .....	10	....	4(8pf)	8(+71)	8(+120uf)
Sheep, mountain .....	4	....	....	2(11)	3(10uf)

\* Apparently all were partly to fully engorged nymphs when collected, and molting to adults took place after collection at Virginia Dale, Colo., Dec. 15, 1940.

mology of Montana, and the Bureau of Entomology and Plant Quarantine, there is still much to be learned regarding the interrelations of the parasite, its hosts, and the diseases it carries in different areas in which it occurs.

### Distribution

The distribution of the Rocky Mountain spotted fever tick has been discussed and maps presented by a number of authors—Bishopp (1911b), Hooker, Bishopp and Wood (1912), Hunter and Bishopp (1911), and Cooley (1911, 1938). Information regarding its distribution in Canada has been referred to by numerous writers, particularly Hadwen (1923), and Twinn (1932, p. 164).

In general, this species ranges from the western counties of Nebraska and the Black Hills of South Dakota to the eastern slopes of the Cascades, and from the

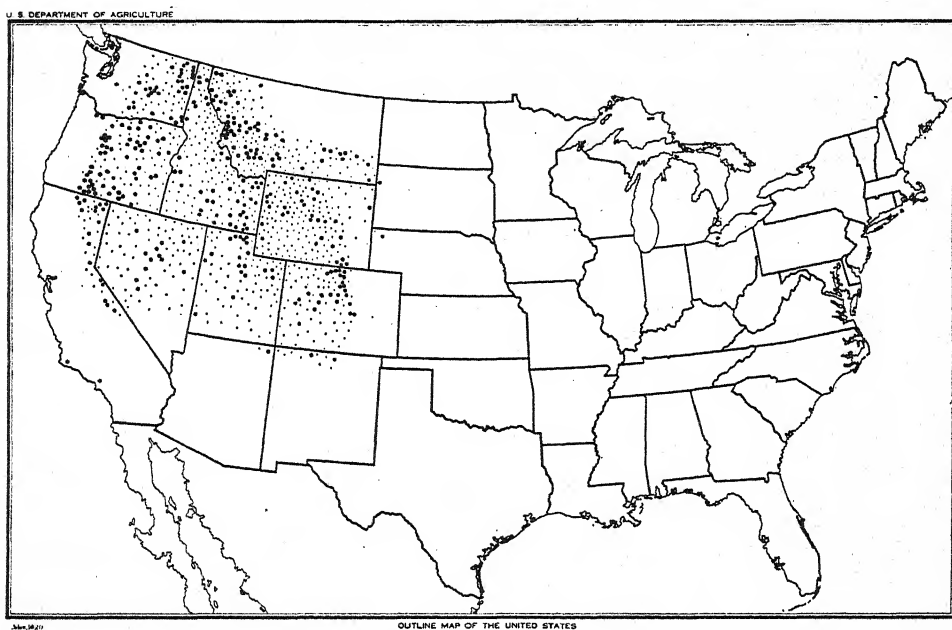


FIG. 6. Distribution of *Dermacentor andersoni* in the United States. Large dots indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

northern portions of Arizona and New Mexico well into British Columbia and Manitoba (Fig. 6). As with most species of ticks, the abundance varies greatly in different areas, and there are included in the "area of probable distribution" on the map many districts of considerable size where usually the tick is a rarity. The species is especially abundant in rough, cut-over, mountainous areas, and in sagebrush country near streams, because such conditions provide favorable cover and abundant hosts for all stages of this tick.

Two records from western California (Santa Barbara and Whittier) are of interest in that they involve localities well outside the normal range of the species, and one of these (from Whittier) was of a one-third-engorged female from a patient in a hospital. This patient had not been away from that vicinity or out into areas likely to be infested. All these occurrences were of single specimens, undoubtedly



accidental introductions, and certainly do not represent established infestations. We have three records each from Bonneville, Ore., and Portland, Ore. The records from Bonneville indicate that the tick is present there in nature. Two of these lots (1 male and 2 males, respectively) were from Works Progress Administration workers, and the third lot consisted of 1 male and 1 female found attached to children. The collections from Portland do not indicate so strongly that the tick is normally present there. One of them (1 male and 1 female) was from children who had come from Pendleton, Ore., where the tick is abundant, and one was from a butcher who handled calves slaughtered and sold with the hides on. These animals may have originated in tick territory to the east.

This tick is abundant in southeastern British Columbia and throughout Alberta and Saskatchewan. Gibbons (1939) says, "There are also isolated records of its occurrence in Manitoba."

It is noteworthy that there is a definite overlapping of distribution of this species and *Dermacentor variabilis* in eastern California, eastern Montana, western North and South Dakota, and western Nebraska. It also appears that both species may be found in certain restricted areas in southern Oregon.

#### Hosts

Intensive studies of the host relations of *Dermacentor andersoni* have been made in the Bitter Root Valley of Montana and in the vicinity of Powder River, Mont.

Rather extensive collections also have been made in several of the infested states. This species does not attack birds or cold-blooded animals, but it is found on a great variety of mammals. The immature stages attach mainly to the smaller wild rodents that range in size from shrews and jumping mice to woodchucks and rabbits. They are found only rarely on the larger mammals and man. We have no records of the attachment of larvae to man, but have two clear-cut records of nymphs attached to children and adults.

The relative importance of different animals as hosts of the adults varies in different localities. It depends much on the abundance of the different host species. Horses and cattle are often very heavily infested, and, in general, may be regarded as the most important hosts of the adults.

The adults are found attached to animals soon after the snows begin to disappear, reach a maximum abundance during April and May, and decline in numbers about midsummer. Our earliest records are from northern Colorado. One male was collected on a calf January 6, 1918, at Virginia Dale by B. G. D. Bishopp. In the same locality a male was collected on February 15, 1916, and another on February 23, 1916, each on man. In Estes Park, Colo., a female was taken on clothing on February 22, 1925. Early adult activity was also observed at Viewpoint, Ore., where H. H. Hatch collected a male on man February 3, 1913, and specimens of both sexes on cattle on March 8, 1913.

The earliest record we have for the Bitter Root Valley is a male taken on clothing on March 11, 1910. Nymphs begin to appear on hosts early in April, but larvae do not appear to be found on hosts until the first half of June. Adults become scarce on hosts after August 1, but there are a number of records of the collection of occasional specimens in September and October. Most of the late records are from

Oregon, and the latest one was a male attached to a man at Lake, Ore., November 22, 1920.

Records of the Bureau of Entomology and Plant Quarantine are given in Table 6.

TABLE 6.—*Dermacentor andersoni*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Antelope, American pronghorn	1	....	....	1(14)	1(2sp)
Ass	4	....	....	4(+8)	4(+6uf)
Badger	1	....	....	1(1)	....
Bear	1	....	....	1(4)	1(9sp)
Bobcat	1	....	....	1(1)	....
Cat, domestic	3	....	2(3)	1(1)	....
Cattle	95	....	71(3)	73(+350)	581(+422)
Chipmunk, buff-bellied	11	7(173up)	6(10up)	....	....
Chipmunk, Colorado	1	1(2)	....	....	....
Chipmunk ( <i>Eutamias</i> spp.)	15	8(432uf)	12(59uf)	....	....
Chipmunk, painted	1	....	1(1s)	....	....
Cony (including Taylor and Rocky Mountain pikas)	2	....	2(7uf)	1(1)	....
Coyote	1	....	....	1(15)	1(16up)
Coyote, Great Basin	1	....	....	1(1)	....
Deer, Rocky Mountain mule	4	....	....	3(7)	3(5uf)
Dog	21	....	....	14(+25)	16(+32uf)
Elk	2	....	....	2(2)	....
Goat	1	....	....	....	1(4us)
Goat, mountain	4	....	....	4(+8)	3(+5uf)
Ground squirrel ( <i>Callospermophilus</i> spp.)	4	1(17sf)	4(7up)	....	....
Ground squirrel (chickaree), Douglas	3	1(13pf)	3(3uf)	....	....
Ground squirrel ( <i>Citellus columbianus</i> subsp.)	64	19(514uf)	53(+801uf)	....	....
Ground squirrel ( <i>Citellus</i> sp.)	3	1(13pf)	3(3uf)	....	....
Ground squirrel, gilded	2	1(5sp)	2(8sf)	....	....
Ground squirrel, little gray	1	....	1(2)	....	....
Ground squirrel, Montana mantled	16	6(67uf)	16(122pf)	....	....
Ground squirrel, Oregon	1	....	1(3s)	....	....
Ground squirrel, Wyoming	3	....	1(1s)	....	....
Hog	3	....	....	2(11)	2(10sp)
Horse	353	....	....	235(+2000)	332(+2574uf)
Man	270	....	2(2p)	189(+513)	204(614 up)
Mouse, Cary meadow	1	....	1(5sp)	....	....
Mouse ( <i>Microtus</i> sp.)	2	....	2(9uf)	....	....
Mouse, Rocky Mountain jumping	1	1(1p)	....	....	....
Mouse, white-footed	4	1(4uf)	4(4us)	....	....
Mule	5	....	....	5(+9)	5(+13uf)
Pocket gopher, Nevada	2	2(sp)	....	....	....
Porcupine	13	....	....	13(+50)	12(57uf)
Porcupine, yellow-haired	3	....	....	3(31)	2(30uf)
Rabbit, Black Hills cottontail	2	1(3)	1(1)	1(1)	....
Rabbit, black-tailed jack	1	....	1(3pf)	....	....
Rabbit, cottontail	10	3(59uf)	9(28uf)	....	2(2pf)
Rabbit, cottontail ( <i>S. nuttalli</i> )	1	....	1(1f)	....	....
Rabbit, Idaho pygmy	1	....	1(21uf)	....	....
Rabbit, jack	6	....	2(2)	4(19)	3(14up)
Rabbit, Rocky Mountain snowshoe	2	....	....	1(1)	1(1f)
Rabbit, snowshoe	3	....	1(1s)	1(1)	2(2pf)
Rabbit, Washington jack	7	....	1(2p)	3(10)	....
Rabbit, white-tailed jack	1	1(3)	....	....	....
Sheep	8	....	....	6(+37)	6(+32uf)
Squirrel, Richardson red	28	18(+644uf)	22(169uf)	....	....
Woodchuck ( <i>Marmota flaviventris</i> )	25	1(8us)	26(194uf)	....	....
Woodchuck, pallid yellow-bellied	1	....	....	....	1(1u)
Woodchuck, yellow-bellied	2	....	2(16sf)	....	....
Wood rat, bushy-tailed	9	5(65uf)	6(13uf)	....	....
Wood rat, western bushy-tailed	1	1(12sf)	1(34sf)	....	....

### *Dermacentor nigrolineatus* Pack.

#### The Brown Winter Tick

As explained in the discussion of *Dermacentor albipictus*, that species is regarded by some as identical with *D. nigrolineatus*. In this article these forms are considered as distinct. *D. nigrolineatus* remains on the host during each of its two molts. It is a cool-weather species, being found on hosts only during fall, winter, and spring.

This tick is a pest of considerable importance on horse and cattle in parts of the Southwest; and antelope, deer, and elk are sometimes heavily infested. It is not known to carry any disease, and we have no record of its attachment to man.

### Distribution

This form is most abundant in the southern portions of the country, especially in the arid part of Texas and New Mexico (Fig. 5), and in northern Mexico. It is occasionally found also in considerable numbers on horses and cattle in the more humid parts of the South.

### Hosts

The host relations of this species are similar to those of *Dermacentor albipictus*. In the Southwestern States it is very abundant on horses, which appear to be the preferred host. It is also commonly found on mules, cattle, deer, and antelope.

Adults begin to appear on hosts about October 1. For instance, we have records from Akin County, Minn., September 28, 1937, and Summerville, S. C.,

TABLE 7.—*Dermacentor nigrolineatus*, *D. nitens*, *D. occidentalis*, and *D. parumapertus*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
<i>Dermacentor nigrolineatus</i>					
Antelope .....	5	.....	2(4sf)	2(23)	5(122uf)
Ass .....	1*	.....	.....	.....	.....
Bison, American .....	3	.....	2(2pf)	1(5)	1(8p)
Cattle .....	48	1(+)	17(+170uf)	16(91)	42(+265uf)
Deer, mule (including some skins) .....	3	.....	.....	1(3)	3(6us)
Deer, Virginia .....	74	5(13pf)	34(+640uf)	52(+283)	50(+295uf)
Mule .....	3	.....	.....	1(1)	3(8pf)
<i>Dermacentor nitens</i>					
Cattle .....	5	.....	1(3pf)	3(+4)	2(+1f)
Deer, Virginia .....	1	.....	†	†	†
Goat .....	1	.....	1(1p)	†	†
Horse .....	20	3(†uf)	† uf)	13(+84)	10(† uf)
Mule .....	7	.....	3(†)	6(†)	4(† uf)
<i>Dermacentor occidentalis</i>					
Ass .....	1	.....	.....	† uf	† uf
Cattle .....	42	.....	.....	32(+179)	37(+235uf)
Chipmunk, Merriam .....	1	1(2s)	1(6sf)	.....	.....
Deer, Rocky Mountain mule ..	11	.....	.....	12(17)	7(11uf)
Dog .....	3	.....	.....	2(3)	3(4u)
Ground squirrel .....	1	.....	.....	1(1)	.....
Horse .....	41	.....	.....	27(106)	39(+115uf)
Man .....	44	.....	.....	29(59)	32(81us)
Mouse, Gambel white-footed ..	2	.....	1(1p)	.....	.....
Mule .....	10	.....	.....	5(8)	7(16uf)
Rabbit .....	1	.....	1(2s)	1(13)	1(12uf)
Sheep .....	1	.....	.....	1(1)	.....
Wood rat, large-eared .....	1	.....	1(2sp)	.....	.....
Wood rat, Streater .....	1	1(1)	.....	.....	.....
<i>Dermacentor parumapertus</i>					
Cattle .....	1	.....	.....	.....	1(6sf)
Mouse, pocket .....	1	1(50uf)	.....	.....	.....
Mouse, Tejon pocket .....	4	2(74sf)	4(7sf)	.....	.....
Rabbit .....	22	.....	5(7uf)	20(136)	20(98uf)
Rabbit, antelope jack .....	6	1(5f)	.....	5(+26)	5(+29uf)
Rabbit, California jack .....	6	.....	3(42uf)	4(12)	5(17uf)
Rabbit, Colorado desert jack ..	1	1(20sp)	1(7uf)	.....	.....
Rabbit, cottontail .....	18	5(27uf)	8(32uf)	5(+19)	7(+17uf)
Rabbit, Great Plains jack .....	2	2(26uf)	1(2f)	1(1)	.....
Rabbit, jack .....	93	6(65sp)	24(259uf)	56(571)	66(421uf)
Rabbit, pygmy .....	1	.....	.....	1(1)	1(18)
Rabbit, white-tailed jack .....	1	.....	.....	1(2)	.....
Rat, Mohave kangaroo .....	1	.....	1(3sf)	.....	.....
Rat, Richardson kangaroo .....	1	1(17us)	.....	.....	.....

\* All stages. † Several. ‡ Many.

October 1, 1933. Seldom are they present on hosts after the middle of April (Warren County, Miss., April 15, 1921, and Alamogordo, N. Mex., April 12, 1923 are records of late occurrence). They have been collected on animals throughout the winter. Bureau records are shown in Table 7.

*Dermacentor nitens* Neum.

The Tropical Horse Tick

The tropical horse tick is a pest of distinct importance in extreme southern Texas and southward through the warmer parts of Mexico, Central America, and in the West Indies. It remains on the host for each of its two molts and is most commonly found in the ears of horses, asses, and mules. Although its mouthparts

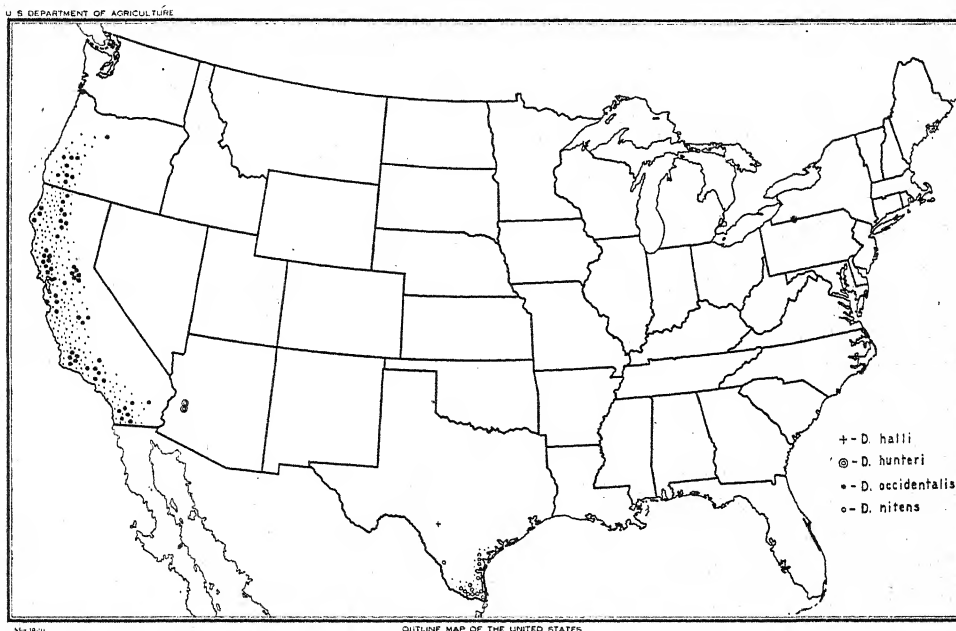


FIG. 7. Distribution of *Dermacentor halli*, *D. hunteri*, *D. occidentalis*, and *D. nitens* in the United States.

are short it frequently sets up considerable inflammation, and screwworm infestations may follow. The inside of the ears of hosts frequently becomes closely packed with ticks in all stages and with molted skins and tick excrement which produces a nauseating odor. Infested animals are sensitive when the ears are touched, and putting on halters and bridles becomes difficult.

*Distribution*

The types of the species came from Jamaica and Santo Domingo. The tick is abundant in the southern part of Texas and along the east coast of Mexico. It is also abundant in the West Indies. The Bureau collections contain specimens from Jamaica, Puerto Rico, Cuba, Dominica, Haiti, Saint Thomas, and Saint Croix, also from Guatemala, Costa Rica, Honduras, Panama, and the Canal Zone in Central America. The species has also been recorded from Trinidad and Colombia.



The distribution in the United States is indicated on the accompanying map (Fig. 7). The published record (Banks, 1908) of the occurrence of the species in Arizona is probably based on specimens introduced on horses shipped into that State. Corpus Christi, Nueces County, Tex., is the northernmost locality in which it occurs commonly. Although a single nymph was collected at Kerrville, Tex., on a deer skin and reported by Hooker, Bishopp, and Wood (1912) as apparently of this species, subsequent extensive collecting in that area has failed to reveal its presence. In addition to this collection in Kerr County, the Bureau collection contains specimens from the following Texas counties: Cameron, Hidalgo, Nueces, San Patricio, Webb, and Willacy.

The fact that this tick remains on a host for its molts and requires 26 days, or longer, for its development on a host gives ready opportunity for it to be shipped long distances. There is no evidence, however, that it has become established in any of the more northern localities in this country to which infested animals have undoubtedly been shipped.

#### *Hosts*

Horses, asses, and mules are preferred hosts of the tropical horse tick. A high percentage of these hosts in the Brownsville, Tex., area and along the east coast of Mexico are infested. Tate (1941, p. 19) found 57 per cent of 131 horses examined in various parts of Puerto Rico to be infested and about 15 per cent of the goats and sheep.

The inside of the external ear is the preferred place of attachment, but it is not very unusual to find this species on the outside of the ears, on the top of the head, in the mane, or occasionally on the body.

This tick in all stages is found on hosts throughout the year, though it is probably more abundant in Texas during the fall, winter and spring.

Bureau collection records in the United States are given in Table 7.

The collection also contains many lots from the West Indies, Mexico, and Central America from ass, goat, horse, mule, and sheep.

#### *Dermacentor occidentalis* Marx

##### The Pacific Coast Tick

The Pacific Coast Tick is a common wood tick in western California and southwestern Oregon. Although it frequently attaches to man, it has not been definitely shown to transmit Rocky Mountain spotted fever in nature, although Parker, Phillip, and Jellison (1933) found it to be a transmitter of the fever under experimental conditions; and there appears to be no reason why it should not serve as a vector among both wild rodents and man. Parker, Brooks, and Marsh (1929) demonstrated the occurrence of tularemia organisms in adult ticks received from cattle in California, and *Bacterium tularense* has been found repeatedly in adults of this species in nature. Anaplasmosis of cattle has been transmitted by larvae and nymphs in experiments by Herms and Howell (1935).

#### *Distribution*

The type locality of the Pacific Coast tick is Occidental, Calif. The tick is rather abundant in the Coastal Ranges and Cascade Range in California and southern

Oregon, and less abundant in the San Joaquin and Sacramento Valleys and on the west side of the Sierra Nevada (Fig. 7). Reports of the occurrence of this tick in Texas, New Mexico, and Arizona are probably incorrect; at any rate it does not appear to be established in any of those States. Since this species is present in abundance in southern California, it undoubtedly occurs in Lower California. Our northernmost record is from Ashwood, Ore. (2 nymphs, 13 males, 12 females from rabbit, June 16, 1910). Chamberlain (1937) reported a collection nearly as far north but on the west side of the Cascades at Yachats, Lincoln County, Ore.

### Hosts

Deer was the host of the type specimen. This animal is an important host of the adults, which sometimes are present on it by the hundreds. Cattle and horses are important hosts of the adults, and in a few instances nymphs have been taken on these animals. Larvae and nymphs engorge readily on bovines—also on rabbits and guinea pigs according to Hooker, Bishopp, and Wood (1912).

Kohls (1937) made a study of the host relations of the immature stages of the Pacific Coast tick in Oregon and California and listed 26 hosts. He concludes that, because of their abundance and general distribution in regions infested by this species, ground squirrels (*Citellus douglasii* and *C. beecheyi*) and white-footed mice (*Peromyscus* spp.) are of major importance. He also concludes that the following are important: Wood rats (*Neotoma* spp.), bush rabbits (*Sylvilagus bachmani*), cottontail rabbits (*S. audubonii*), jack rabbits (*Lepus californicus*), pocket mouse (*Perognathus* sp.), and chipmunk (*Eutamias* sp.). He reports that a single nymph was found attached to his arm. Chamberlain (1937) lists lizard as a host in Oregon.

The immature stages appear to be most abundant on hosts in the spring and summer. Our collection contains adults taken every month except September, thus indicating its presence on hosts throughout the year. Adults reach a peak of abundance in April and May. The Bureau host records are shown in Table 7.

### *Dermacentor parumapertus* Neum.

#### The Rabbit *Dermacentor*

The authors consider the form *Dermacentor parumapertus marginatus* Banks as indistinguishable as an entity from *D. parumapertus*.

This species has not been shown to be of any particular economic importance, as it confines its attack largely to rabbits. It may well play a part, however, in transmitting tularemia, Rocky Mountain spotted fever, and other diseases among these animals.

The types were recorded from "man and in a chicken house," Lakeside, Calif. Banks' types of *Dermacentor parumapertus marginatus* were from jack rabbit, Mesa, Ariz.

### Distribution

The rabbit *Dermacentor* is fairly abundant in the Southwestern States west of the 100th meridian and in the arid portions of northern Mexico. It appears to thrive in areas of light rainfall and is found in association with rabbits under extreme desert conditions. Although it has been collected in timbered sections of the

West, it is more typically a desert species. It is most abundant in western Texas and southern New Mexico, Arizona, and California. It is fairly abundant in some parts of Utah and Nevada (Fig. 8).

The seasonal occurrence of this tick is interesting but not well understood. The immature stages are found on hosts mainly during the cooler months, September to May. The adults are present on hosts throughout the year in the southern range of the species although they are more abundant in summer. Often large numbers of females are found with few or none showing appreciable engorgement; again, considerable numbers of engorging females are in evidence. This has suggested a possible relation between engorgement and seasonal rainfall.

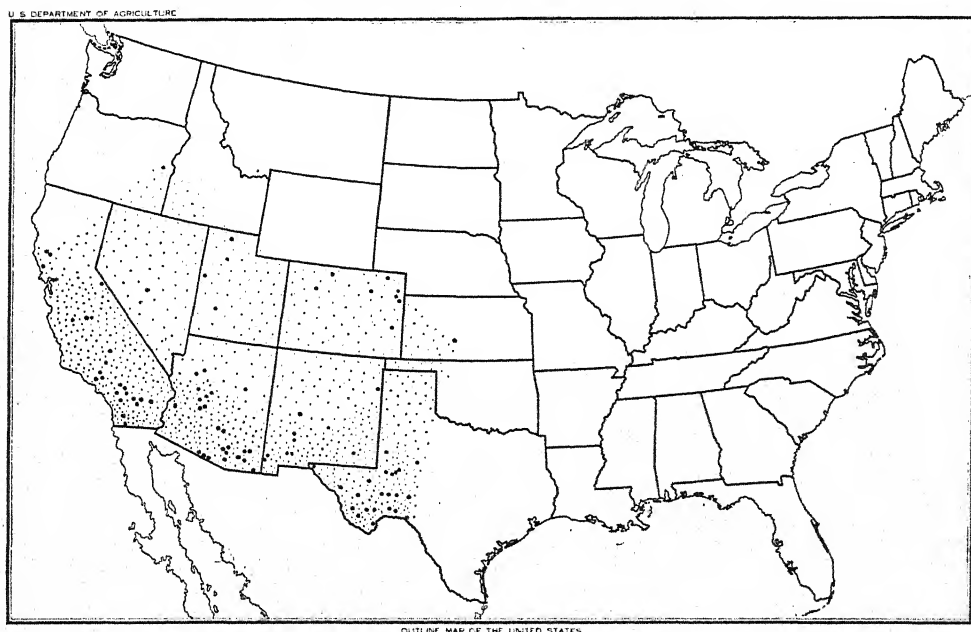


FIG. 8. Distribution of *Dermacentor parumapertus* in the United States. Large dots indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

#### Hosts

Jack rabbits are the principal hosts of the larvae, nymphs, and adults, partly because of the greater abundance of these rabbits in much of the region inhabited by the tick. Other kinds of rabbits are excellent hosts, however, and some are found to be heavily infested. Our records show no collections on man or domestic animals except one lot from cattle collected by R. W. Wells at Wilcox, Ariz., July 29, 1918. Experimentally, adults of this species feed readily on cattle. Boynton and Woods (1933) have recorded this species from deer, and Cooley (1938) has seen specimens taken from coyote in California.

Bureau records of hosts are given in Table 7.

#### *Dermacentor variabilis* (Say)

##### The American Dog Tick

The American dog tick is the principal transmitter to man of Rocky Mountain

spotted fever in the Mississippi Valley, eastward to the Atlantic and southward to the Gulf of Mexico, and including Texas. It plays a small part in the transmission of tularemia and is a source of much annoyance to man and dogs. The experimental transmission of anaplasmosis has been reported by Rees (1934). Dogs are often so heavily infested as to become emaciated, cross, and unsightly. Some districts along the Atlantic Coast have been so adversely affected by the abundance of this tick as to impair their popularity as summer recreational areas.

### Distribution

The American dog tick is one of our most widely distributed ticks, but its abundance varies greatly in different localities. As shown on the accompanying map (Fig. 9) it is found throughout the United States with the exception of Washington

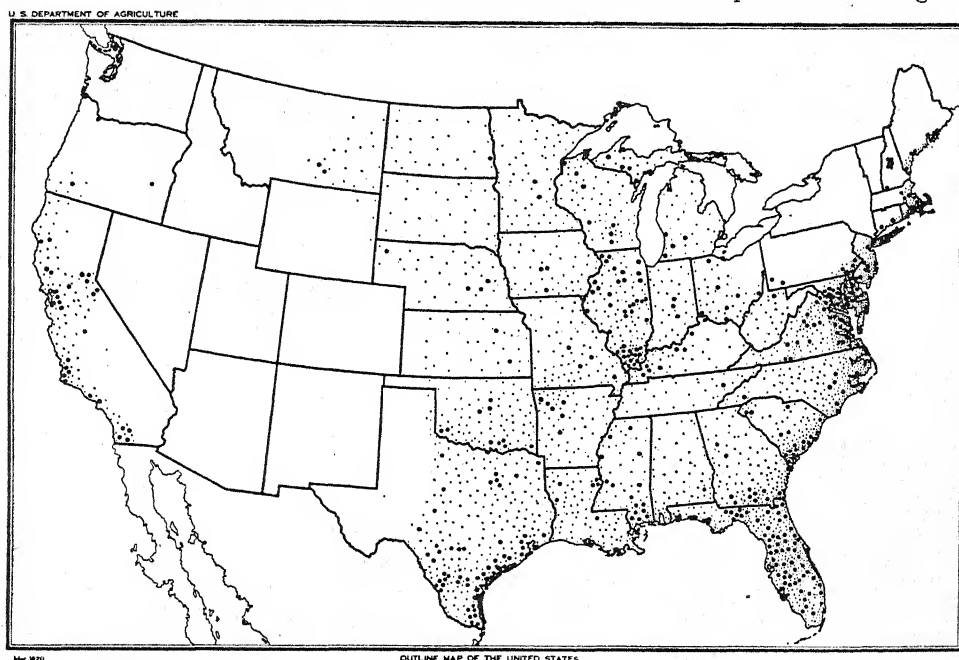


FIG. 9. Distribution of *Dermacentor variabilis* in the United States. Large dots indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

and the Rocky Mountain and Intermountain regions. This species is commonly met in the southern provinces of Canada, and is found in Mexico. It has been reported from Alaska, and there is one record of its occurrence in each of the States of Colorado, Arizona, and New Mexico, but it is doubtful if the occurrence is normal in these localities.

This tick appears to be favored by a humid atmosphere, and this probably is a factor in governing its distribution and abundance. It is very numerous on islands along the east coast of the United States, on Cape Cod, Long Island, and southward along the coast. It is common also in the Gulf Coast States, especially Florida and Texas. As pointed out by Bishopp and Smith (1938), it is less abundant on the north side than on the south side of Cape Cod, and there are few north of Plymouth,



Mass., or inland beyond Middleboro and Taunton, Mass. Since this species is dependent on small, wild rodents as hosts for the immature stages, the distribution and abundance of these animals is an influencing factor on the incidence of the tick, but this does not account for the absence of the species inland in Massachusetts and in many other areas. High humidity appears to be an important factor favoring this species. Heavily-forested areas have fewer ticks than grassy and brush-covered ones, probably on account of the relative scarcity of small animal hosts and appropriate ground cover.

### Hosts

The adults prefer the dog as a host, and although man is frequently attacked, he is not among the preferred group. The immature stages engorge mainly on small rodents, especially mice; of these, meadow mice are preferred, perhaps on account of their habits. In the Southern States ticks in all stages may be found on hosts throughout the year, though they are usually more abundant in the spring. The tendency for the adults to attach to hosts in the spring is more pronounced in the Central and Northern States. In fact, in the North they are uncommon on hosts

TABLE 8.—*Dermacentor variabilis*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Ass	1	.....	.....	1	1(f)
Badger	6	.....	.....	4	6(6uf)
Bear	1	.....	.....	*	*
Cat, domestic	5	.....	2(p)	1	2(f)
Cattle	35	.....	1(f)	18	25(uf)
Coyote	26	.....	.....	16	19(pf)
Deer	3	.....	.....	2	2(uf)
Dog	277	.....	3(pf)	147	218(uf)
Fox	3	.....	.....	2	1(f)
Goat	1	.....	.....	1	.....
Gopher, southern pocket	1	1(u)	.....	.....	.....
Hog	20	.....	.....	8	17(uf)
Horse	20	.....	.....	10	14(uf)
Leopard-cat	1	.....	.....	.....	1(f)
Man	113	.....	.....	48	70(up)
Mexican lion	1	.....	.....	.....	1(u)
Mole	1	1(u)	.....	.....	.....
Mountain lion	1	.....	.....	1	1(f)
Mouse	10	6(uf)	6(uf)	.....	.....
Mouse, Baird's	1	1(u)	.....	.....	.....
Mouse, Cooper's	1	1(f)	.....	.....	.....
Mouse, house	3	3(u)	.....	.....	.....
Mouse (including white-footed and cotton)	79	80(uf)	25(uf)	.....	.....
Mouse, jumping	2	1(f)	1(u)	.....	.....
Mouse, meadow	20	18(uf)	19(uf)	.....	.....
Mouse, pine	11	4(up)	8(uf)	.....	.....
Mule	1	.....	.....	.....	1(u)
Muskrat	3	2(3Spf)	2(2pf)	1(10)	1(6pf)
Opossum	20	.....	1(1)	18	15(uf)
Peccary	3	.....	.....	2	2(f)
Porcupine	2	.....	1(1p)	1(2)	1(s)
Rabbit, black-tailed jack	1	1(3s)	.....	.....	.....
Rabbit, cottontail	34	27(uf)	14(uf)	2	1(s)
Rabbit, domestic	1	.....	.....	1(2)	1(s)
Rabbit, Florida marsh	2	1(p)	2(p)	.....	.....
Raccoon	13	.....	.....	11	9(uf)
Rat, cotton	13	10(52uf)	9(27sf)	.....	.....
Rat, Norway	5	4(up)	1(p)	.....	.....
Rat ( <i>Rattus</i> sp.)	1	1(1u)	1(1f)	.....	.....
Rat, rice	15	13(+ 66uf)	8(+ 25sf)	.....	.....
Sheep	7	.....	.....	5	5(uf)
Shrew, short-tailed	2	2	1	.....	.....
Skunk	5	.....	.....	5	3(us)
Squirrel (including fox, gray and red)	19	1(f)	7	8	8(sp)
Weasel	3	.....	.....	1	2
Wildcat (lynx)	10	.....	.....	8	8(uf)
Wolf	4	.....	.....	4	3
Woodchuck	3	.....	.....	5	6(sp)
Wood rat ( <i>Neotoma</i> sp.)	1	.....	1(p)	.....	.....

\* Several.

after August 1 although stragglers may be found up to the occurrence of freezing weather. The adults first appear in the spring on hosts in Maryland between March 15 and April 15, and the height of abundance comes in the latter part of May or early in June. Larvae and nymphs are found on meadow mice throughout the winter in the vicinity of the District of Columbia. Farther north, in New Jersey and Massachusetts, this is not true. Here the immature stages occur on hosts throughout the spring, summer, and fall, but the larvae are much less abundant in the spring. The relative frequency with which this tick, in its different stages, is taken on various hosts is indicated by the collection records of the Bureau of Entomology and Plant Quarantine in Table 8.

### *Dermacentor hunteri* Bishopp

#### *Distribution*

*Dermacentor hunteri* appears to have a very limited distribution. It has been taken by us only among the isolated mountain peaks of southwestern Arizona (Fig. 7).

#### *Hosts*

Mountain sheep (originally referred to as *Ovis mexicanus* but later identified as *O. canadensis gaillardi* Mearns) is the type host and we have no collections from other hosts. The type of the species was chosen from a lot of 23 males and 12 females collected at Quartzside, Ariz., on September 2, 1911, by Mr. George Hutson. Several other lots of males and females (in various stages of engorgement) were collected during July, August, September, November, and December in the same locality and at altitudes of 1,500 to 2,000 feet.

### *Haemaphysalis chordeilis* Pack.

#### The Bird Tick

The bird tick appears to have a rather wide distribution in the United States, but only occasionally does it assume economic importance. Losses caused by it have been observed among turkeys and certain game birds. It may sometimes have an adverse effect on other wild birds, also. There is some confusion in the taxonomy of this species. Nuttall et al. (1915) regard *Haemaphysalis chordeilis* Pack. as a synonym of *H. cinnabarina* Koch. We are inclined to think these forms distinct, and the records presented herein are based on the restricted application of the name.

#### *Distribution*

This is an American species which is found in the eastern and southern parts of the United States and in eastern Canada. If the broader interpretation of the species as mentioned above is applied, the western states and western Canada would also be included. Our records seem to indicate that it is most abundant in the Gulf Coast and Atlantic Coast regions (Fig. 10). Bureau records do not indicate its presence in the Rocky Mountain and intermountain regions. Dr. W. A. Riley informs us that he received a report of a severe infestation of what he then called *H. cinnabarina* on turkeys in northern Minnesota. Apparently the species does not thrive in the drier portions of the country. No doubt the immature stages and perhaps the adults have been collected in many instances and considered as *Haema-*

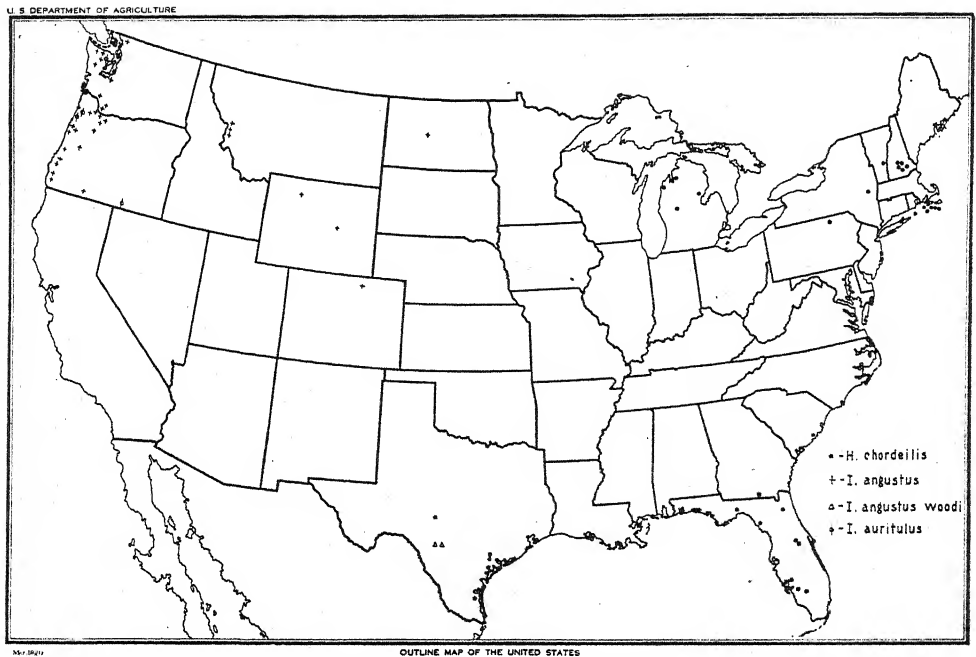


FIG. 10. Distribution of *Haemaphysalis chordeilis*, *Ixodes angustus*, *I. a. woodi*, and *I. auritulus* in the United States, as indicated by collections of the Bureau of Entomology and Plant Quarantine.

*physalis leporis-palustris*, and more intensive collecting will show the species to be more widely distributed than shown in Fig. 10.

### Hosts

Birds are the preferred hosts of all stages of this species, hence the common name "bird tick." Grouse and meadowlarks appear to be most freely attacked. The infestation of turkeys by this tick has been recorded by Banks (1908). The writers have obtained information to the effect that the bird tick often causes the death of poults along the coast in Connecticut, and that young grouse reared and

TABLE 9.—*Haemaphysalis chordeilis*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Blackbird, red-winged ..	1	.....	1(2f)	.....	.....
Cow .....	1	.....	.....	.....	1(1p)
Grouse, ruffed (including <i>Bonasa umbellus</i> ) ...	16	7(31sf)	10(24sf)	4(26)	10(18sp)
Hawk, marsh .....	1	.....	1(2)	.....	.....
Horse .....	1	.....	.....	.....	1(1p)
Jackdaw .....	5	5(39uf)	.....	.....	.....
Man .....	1	.....	.....	.....	1(1)
Meadowlark .....	24	12(+ 110uf)	16(147uf)	9(26)	2(5p)
Meadowlark, southern ..	21	10(+ 194 uf)	13(+ 48sf)	6(21)	4(9sp)
Quail .....	4	1(15uf)	2(21uf)	.....	1(9uf)
Sheep .....	1	.....	.....	.....	1(1u)
Sparrow, Florida .....	1	1(1f)	1(2f)	.....	.....
Sparrow, Savannah .....	2	.....	2(2sp)	.....	.....
Sparrow, Wakulla sea- side .....	1	.....	1(1u)	.....	.....
Towhee .....	1	1(1f)	.....	.....	.....
Turkey .....	2	1(+ 1f)	.....	.....	2(+ 2pf)
Wren, Florida .....	1	1(1s)	.....	.....	.....

released in late summer on Fishers Island, N. Y., often become heavily infested, and that the mortality is rather high. That there is some tendency for this species to catch hold of clothing is indicated by the fact that C. N. Smith collected 16 males and 17 females on clothing and on a cloth drag at Woods Hole, Mass., April 23, 1941.

All specimens of the bird tick collected in the South (Florida, Georgia, and Texas) were taken during late fall, winter, and early spring and those in the North were collected during the spring, summer, and fall.

Other Bureau of Entomology and Plant Quarantine records are to be found in Table 9.

*Haemaphysalis leporis-palustris* Pack.

The Rabbit Tick

The rabbit tick is one of the most common and widely distributed species of ticks in North America. The fact that it does not ordinarily attack domestic animals and that it never attacks man cause it to be ranked as of little economic importance. However, since it becomes so abundant on wild rabbits and certain birds as to weaken if not kill them, and since it is capable of carrying tularemia and Rocky Mountain spotted fever from animal to animal, it should be placed among the species of importance to man.

Hooker, Bishopp, and Wood (1912, p. 96) record the collection of 1,033 specimens of this tick, many of which were engorged females, on 2 snowshoe hares. Greene, Bell, and Evans (1938) have reported infestations of this hare reaching an average of almost 5,000 ticks per animal in September and October 1933, and in excess of 4,000 per animal during the late summer of 1935. The authors have observed quail and meadowlarks so heavily infested that they were emaciated and they believe that some, especially the young birds, may be killed by gross infestation. This tick occasionally attaches to chickens. The authors have one record of 19 larvae picked from the head and throat of a chick in 24 hours at Harvel, Ill., September 5, 1922.

*Distribution*

The rabbit tick is widely distributed in North America, from Alaska and the Southern Canadian Provinces southward well into Mexico. Collections of the Bureau of Entomology and Plant Quarantine contain specimens from every State in the Union except South Dakota, Nebraska, Missouri, Arkansas, Delaware, Kentucky, and West Virginia, and there is no reason to believe that it does not occur in those 7 States. It appears to be least abundant, in proportion to the number of rabbits, in extreme western Texas, in New Mexico, in Arizona, and in northern New England. Although the accompanying map (Fig. 11) indicates that many lots were collected in Texas, this does not mean that the tick is more abundant there than in many other parts of the country.

*Hosts*

Rabbits are the preferred hosts of this tick, particularly of the adult stage; in fact, it is unusual to find adults on any other host. Birds of many kinds serve as hosts of the larvae and nymphs, and, as indicated above, certain ground-inhabiting birds may be very heavily infested. In general, these ticks attach in greatest numbers



to the heads of their hosts. A large percentage of those on birds are to be found on the top and back of the head, and around the eyes and ears. In the case of rabbits, a large percentage of the ticks attach to the ears, around the eyes, and on other parts of the head; occasionally they are found between the toes and on the body.

The rabbit tick in all stages is found attached to hosts throughout the year in the Southern States. Its activity ceases during the colder parts of the year in the more northern States. Green, Bell, and Evans (1938) found 9 ticks on 13 hares during the first week in December 1931; they found none during the second week on 16 hares, but found that immature ticks began to attach to hares in small numbers during the first half of May in 1938. Philip (1938) found the rabbit tick in moderate numbers on the various hares near Seward and Fairbanks, Alaska, during

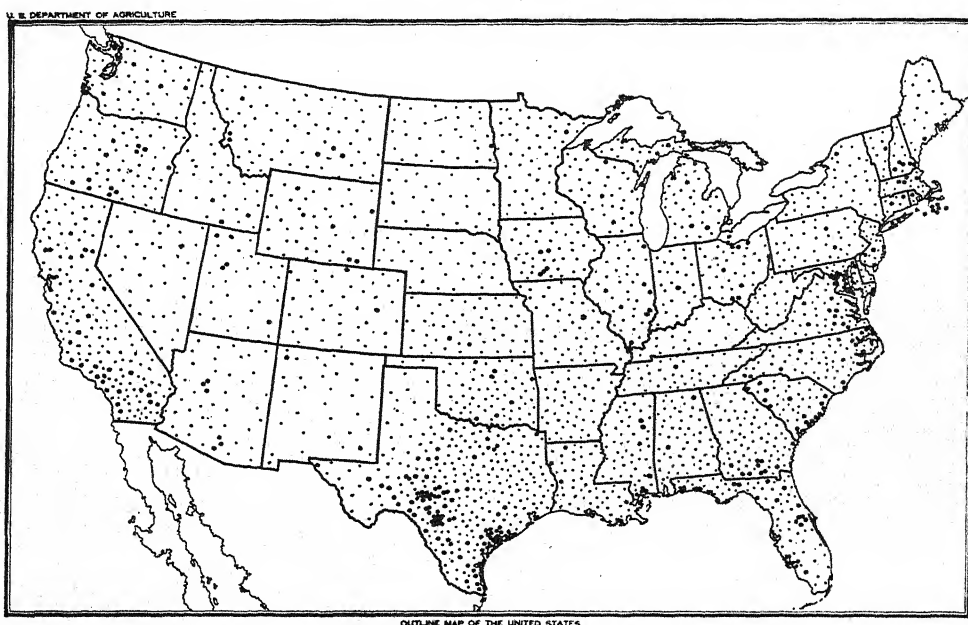


FIG. 11. Distribution of *Haemaphysalis leporis-palustris* in the United States.

June and July. This species is generally more abundant on hosts in spring and fall in the southern portions of the country. It appears to be more or less active during the winter along the Atlantic Coast even as far north as Marthas Vineyard, Mass., as C. N. Smith and M. M. Cole collected 1 nymph and 3 females on a cottontail rabbit on January 29, 1940, and 1 larva and 3 females in January 31, 1941. Nymphs and adults were also taken there in the latter part of March 1941. Collections made by E. B. Marshall in the vicinity of Laurel, Md., during several years show that large numbers of larvae in all states of engorgement and a few nymphs were present on quail through October and November. Joyce and Eddy (1943) examined 56 cottontail rabbits in Iowa between April 16 and December 23, 1941, and found the maximum number per animal (all stages) to occur in August. Adults were most abundant there in spring and absent in October to December, and larvae reached a peak of abundance in August and nymphs in October. The Bureau of Entomology and Plant Quarantine has records of hosts as shown in Table 10.

TABLE 10.—*Haemaphysalis leporis-palustris*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Blackbird, Brewer's . . .	2	3	2		
Blackbird, red-winged . . .	7	3(25sp)	3(4up)		
Bluebird . . . . .	1	1(2p)			
Bluejay . . . . .	10	10(31uf)	4(8sp)		
Bunting, painted . . . . .	1	1(3s)			
Cardinal . . . . .	23	11(47sf)	15(46uf)		
Cat . . . . .	1	1(+p)			
Catbird . . . . .	3	1(+s)	1(+u)		1(+sp)
Chaparral cock . . . . .	15	11(+uf)	12(+sf)		
Chat, yellow-breasted . . . . .	1	1(+p)			
Chicken, domestic . . . . .	5	4(+sf)	2(+sf)		
Cowbird . . . . .	4	4(+uf)	3(+sf)		
Crow . . . . .	2	1(+f)	1(f)		
Dove, mourning . . . . .	1		1(s)		
Fox (faeces) . . . . .	1		1(s)		
Goat . . . . .	2	1(2p)	1(6sp)		
Goldfinch . . . . .	2		1(1)		
Grackle bronze . . . . .	2	2(s)	1(f)		
Grackle, purple . . . . .	2	1(2up)	1(1)		
Ground squirrel . . . . .	1				1(1p)
Ground squirrel striped . . . . .	1				
Grouse, ruffed . . . . .	31	21(many sf)	30(many uf)	1(2)	
Grouse, sharp-tailed . . . . .	1*				
Junco . . . . .	8	5(27sf)	6(7sf)		
Kinglet, ruby-crowned . . . . .	1	1(1u)			
Lark, horned . . . . .	1	1(1f)	1(10sf)		
Lark, prairie horned . . . . .	1	1(2s)			
Magpie . . . . .	2	2(18p)	1(1p)		
Meadowlark, eastern . . . . .	24	20(243uf)	15(42uf)		
Meadowlark, western . . . . .	17	4(28sf)	15(111uf)		
Mockingbird . . . . .	2	2(2pf)			
Mouse, cotton . . . . .	1	1(p)			
Mouse, northern golden . . . . .	2	1(2p)			
Mouse, northern golden nest . . . . .	1				
Mouse, parasitic . . . . .	1		1(1p)		1(1u)
Oven-bird . . . . .	3	3(7)	1(1)		
Owl, buffy prairie . . . . .	1	1(4sf)	1(4sf)		
Prairie chicken . . . . .	3	2(18up)		1(+2)	
Quail (bobwhite, mostly) . . . . .	81	54(uf)	54(uf)	1(+)	3(4up)
Quail, California . . . . .			1(4p)		
Quail, California valley . . . . .	2	2(100sp)	1(2u)		
Quail, Mexican scaled . . . . .	4	4(96up)	3(10up)		
Rabbit, cottontail . . . . .	426	173(+uf)	252(+uf)	189	213(+uf)
Rabbit, domestic . . . . .	3	1(1u)	1(1s)	2	2(sf)
Rabbit, eastern cotton-tail . . . . .	23	1(1s)	13(66sf)	13(47)	16(38sf)
Rabbit, jack (including Washington and Colorado desert) . . . . .	123	13(93 + uf)	48(427 + uf)	90(746 +)	86(501 + uf)
Rabbit, Oklahoma cotton-tail . . . . .	5	3(32sp)	2(17sp)	1(1)	2(6up)
Rail, sora . . . . .	1	1(14uf)	1(2p)		
Rat, roof . . . . .	1	1(1s)			
Redstart . . . . .	1	1(1)			
Robin . . . . .	9†	4(+us)	7(+2uf)		
Sparrow, Bachman's . . . . .	2		2(2uf)		
Sparrow, chipping . . . . .	4	1(1s)	3(4up)		
Sparrow, English . . . . .	1	1(17uf)			
Sparrow, field . . . . .	3	2(2us)	2(2u)		
Sparrow, fox . . . . .	2		2(2s)		
Sparrow, Gambel's . . . . .	1		1(9pf)		
Sparrow, Lincoln . . . . .	1		1(1p)		
Sparrow, Savannah . . . . .	10	2(5pf)	2(7up)		
Sparrow, song . . . . .	33	24(+uf)	21(+uf)	1	
Sparrow, swamp . . . . .	9	5(25uf)	8(uf)		
Sparrow, tree . . . . .	1	1(1p)			
Sparrow, vesper . . . . .	1		1(3pf)		
Sparrow, western field . . . . .	1	1(4s)			
Sparrow, white-crowned . . . . .	5	4(9pf)	2(11sf)		
Sparrow, white-throated . . . . .	16	11(uf)	11(uf)		
Squirrel, fox . . . . .	2		1(2f)		1(2p)
Squirrel, gray . . . . .	1	1(1s)			
Squirrel, pine . . . . .	1	1(21p)			
Starling . . . . .	5	5(27sf)			
Thrasher, brown . . . . .	17	11(uf)	15(uf)		
Thrasher, Crissal . . . . .	1		1(1s)		
Thrasher, sage . . . . .	1	1(1p)			
Thrush, hermit . . . . .	9	9(+uf)	2(2s)		
Thrush, olive-backed . . . . .	6	6(uf)	4(4up)		
Thrush, wood . . . . .	1				1(1s)
Titmouse . . . . .	2	2(51uf)	2(8sf)		
Titmouse tufted . . . . .	2	2(4up)			
Towhee . . . . .	1	11(+36up)	5(26uf)		
Towhee, Alabama . . . . .	1	1(2u)			
Towhee, arctic . . . . .	1	1(10sp)			
Towhee, canyon . . . . .	1		1(1f)		
Towhee, red-eyed . . . . .	3	2(3f)	1(9sf)		
Towhee, white-eyed . . . . .	3	3(14sf)	2(9sp)		
Wood rat, Florida . . . . .	1	1(1)			

\* Several ticks.

† Adult 1.

*Ixodes angustus* Neum.

*Ixodes angustus* has been considered as of no economic importance. However, the fact that it is a general feeder and occasionally attacks man, coupled with the fact that it occurs in areas where Rocky Mountain spotted fever is prevalent, should cause it to be regarded with some suspicion. It does not appear to be abundant in any locality.

*Distribution*

The type female was collected at Shoshone, Idaho. This tick is found most commonly in western Oregon and Washington. Its distribution extends southward into California (Walker Pass and Siskiyou County, Banks, 1908) and northward into British Columbia. Hearle (1938) states that it is one of the commonest species in British Columbia, especially in the coastal district. It has also been recorded from Glacier Bay, Alaska (Banks, 1908). As indicated on the map (Fig. 10), in addition to numerous collections in western Washington and Oregon we have a few specimens from Colorado, Wyoming, Montana, and North Dakota. We also have one collection (4 females, partly engorged) from Quetico, Ontario, one from "Ontario," and one female from the short-tailed shrew taken July 10, 1933, from Gaspé, Quebec. No doubt more intensive collecting of ticks from rodents throughout the Northern States will show the species to be rather widely distributed in that section of the country.

Under the mild climatic conditions prevailing in western Washington and Oregon, adults are found on animals there throughout the year. The collection from Quetico, Ontario, was made on February 1, 1932. Immature stages have not been taken on animals by the authors later than the end of October.

*Hosts*

The wood rat (*Neotoma occidentalis*) is the type host. The species is generally regarded as a rodent parasite. Our records, however, indicate that the adults not infrequently attack the larger mammals, including man. Hadwen (1911) has pointed out that the male is seldom found on hosts but probably stays in the nests of rodents; our records bear this out, as we have only 4 lots (5 specimens) of males taken on hosts. The largest number of specimens, by far, that we have recorded from one host was 62 larvae, 20 nymphs, and 1 female. These were collected from a pika near Florence, Mont., on June 16, 1910, by W. V. King. Bureau of Entomology and Plant Quarantine records are given in Table 11.

*Ixodes angustus* var. *woodi* Bishop

*Ixodes angustus* var. *woodi* is a southwestern form of *I. angustus*. It is of no known economic importance.

*Distribution and Hosts*

Extensive collections of ticks from wood rats have not been made but this form has been taken in limited numbers on that host in southwestern Texas. In the localities where these collections were made, wood rats are very abundant, nesting for the most part in clumps of prickly pear (cactus).

The five lots of this tick in the Bureau collection were all taken from the Baird wood rat, *Neotoma micropus*. Other collection data on these specimens are as

TABLE 11.—*Ixodes angustus*, *Ixodes auritulus*, and *Ixodes brunneus*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
<i>Ixodes angustus</i>					
Bat, little brown	1	.....	.....	.....	1(1s)
Cat	1	.....	.....	.....	1(1p)
Chipmunk, Townsend	2	.....	.....	.....	2(4up)
Dog	3	.....	1(1p)	.....	2(2sp)
Gopher	1	.....	.....	.....	1(1s)
Ground squirrel	2	.....	2(8up)	.....	.....
Ground squirrel, Columbian	1	(1p)	1(2p)	.....	.....
Ground squirrel, Douglas	2	.....	.....	.....	2(2p)
Man	3	.....	.....	.....	3(up)
Mouse, red-backed	1	.....	.....	.....	1(1p)
Mouse, redwood white-footed	7	1(2s)	4(+s)	.....	5(6p)
Mouse, Townsend meadow	2	.....	.....	.....	2(5sp)
Pika	3	3(70p)	3(30up)	.....	3(5p)
Pocket gopher, Columbia	1	.....	1(3sp)	.....	.....
Rabbit	2	.....	1(4sp)	1(2)	2(6sf)
Rat, roof	1	.....	.....	.....	1(2p)
Shrew	1	.....	.....	.....	1(1p)
Shrew, Olympic	1	.....	.....	.....	1(1p)
Shrew, short-tailed	1	.....	.....	.....	1(1s)
Shrew, Trowbridge	1	.....	.....	.....	1(2s)
Shrew, wandering	2	1(2p)	1(2s)	.....	1(1p)
Squirrel, Cascades chickaree	1	.....	.....	.....	1(2p)
Squirrel, redwood chickaree	4	.....	2(6us)	.....	4(24up)
Squirrel, Richardson red	1	.....	1(1p)	.....	.....
Squirrel, western	5	.....	1(2sp)	8(3)	5(18sp)
Wood rat, Colorado bushy-tailed	1	.....	.....	.....	1(1p)
<i>Ixodes auritulus</i>					
Finch, California purple	1	.....	1(2sp)	.....	1(1p)
Grouse, Oregon ruffed	1	1(1p)	1(1p)	.....	2(2p)*
Junco, Shufeldt's	3	.....	1(1)	.....	.....
Mouse, meadow	1	.....	.....	.....	2(2p)
Robin, western	3	.....	.....	1(1)	1(2p)
Sparrow, fox	1	1(1s)	.....	.....	1(2p)
Sparrow, Kodiak fox	1	.....	1(1s)	.....	.....
Sparrow, rusty song	2	.....	.....	.....	2(3p)
Woodchuck	1	.....	1(1p)	.....	.....
Wren	1	.....	1	.....	.....
<i>Ixodes brunneus</i>					
Blackbird	1	.....	1	.....	1(1p)
Blackbird, red-winged	1	.....	.....	.....	2(5sp)
Catbird	2	.....	.....	.....	2(2up)
Grackle, purple	2	.....	.....	.....	.....
Jay, coast	1	1(8sp)	1(2u)	.....	3(3f)
Junco	3	.....	.....	.....	.....
Meadowlark	1	1(1s)	.....	.....	.....
Oven-bird	1	1(3u)	.....	.....	1(1f)
Owl, barred	1	.....	.....	.....	.....
Robin	1	1(5sp)	1(1p)	.....	1(1u)
Robin, western	2	1(1p)	1(2p)	.....	1(1p)
Shrike	1	.....	.....	.....	1(1p)
Sparrow, chipping	1	.....	.....	.....	1(1p)
Sparrow, field	1	.....	.....	.....	1(1f)
Sparrow, golden-crowned	1	1(9sp)	1(3s)	.....	1(1f)
Sparrow, Nuttall's	1	.....	.....	.....	.....
Sparrow, song	1	.....	1(1p)	.....	.....
Sparrow, white-throated	11	2(2pf)	7(11uf)	1	3(3uf)
Starling	1	.....	.....	.....	1(3f)
Swallow, violet-green	1	.....	.....	.....	1(1f)
Thrasher, brown	2	.....	1(1f)	.....	1(1s)
Thrush, hermit	3	1(1s)	1(2sp)	.....	2(2up)
Towhee (including red-eyed and white-eyed)	12	5(12sf)	8(16sf)	.....	2(3p)
Waxwing, cedar	1	.....	.....	.....	1(2p)
Wren, Carolina	2	1(3p)	1(2sp)	.....	.....

follows: Two larvae, 2 nymphs, February 7, 1910, Sabinal, Tex., all slightly to partly engorged. Two females, partly engorged, May 10, 1910, Sabinal, Tex. Three nymphs slightly to partly engorged and 2 females slightly engorged, May 18, 1910, Sabinal, Tex. Three nymphs partly engorged, May 31, 1910, Sabinal, Tex.



The above-mentioned lots were collected by F. C. Pratt and C. T. Atkinson. Two nymphs partly engorged April 6, 1914, Uvalde, Tex., D. C. Parman, collector. See Fig. 10.

*Ixodes auritulus* Neum.

*Ixodes auritulus* is an uncommon species occurring largely on birds. It is of no known economic importance.

*Distribution*

*Ixodes auritulus* was described from specimens taken on birds from the Straits of Magellan and Tierra del Fuego, South America. In North America it has been collected mainly in the extreme northwestern States and the southwestern Provinces of Canada, although we have one nymph that we consider to be this species, collected on meadow mouse at Temagami, Ontario, September 7, 1934. Our collections have come from three localities in Oregon, viz., Tillamook, Netarts, and Adel, with one collection from Spruce, Wash. (Fig. 10). These collections were made in January, February, March, April, July, and November. The species appears to be largely restricted to the moist coastal area.

*Hosts*

As stated above, most of our collections have been from birds, the two exceptions being 1 lot from woodchuck and 1 lot from meadow mouse. Hadwen (1916), who first reported the tick from North America, recorded 1 female from Alaska bald eagle (*Haliaeetus leucocephalus alaskanus* Townsend) and 1 male and 10 larvae from Queen Charlotte jay (*Cyanocitta stelleri carlottae*), Queen Charlotte Islands, British Columbia, June 23, 1910. Philip (1933) records the collection of an engorged female from an English sparrow near Hebo, Ore., August 25, 1932. Hearle (1938) states that in the coastal district of British Columbia it appears to be the commonest tick on blue grouse and willow grouse. Table 11 shows the Bureau records for *Ixodes auritulus*.

*Ixodes brunneus* Koch

*Ixodes brunneus* is an interesting bird tick that occurs in North America, Europe, and Africa. The species confines its attack to birds, and it is unusual to find more than a very few specimens on any one host. Despite the small numbers present, they frequently appear to have a markedly adverse effect on the host. The authors have several records in which birds have been found dead or unable to fly, with an engorged female of this species attached to the neck or head, usually around the eyes. This has occurred in the case of a swallow, a snowbird (*Junco h. hyemalis*), and certain warblers.

In the case of the swallow, the bird was observed to fall from an overhead wire at Harrisburg, Ore., in April 1932. Upon examination the fully engorged female tick was found attached to the neck. The bird died soon after the parasite was removed.

All the birds affected appeared normal except for the presence of the ticks, and it is suggested that the ill effects may be due to a toxin introduced as the ticks become engorged.

*Distribution*

This tick is found largely on migratory birds and apparently remains attached for a considerable period, thus giving an opportunity for wide dissemination. The species

has been recorded from many parts of the world, including England, Italy, France, Nyassaland, and many parts of the United States. The species doubtless occurs in Mexico and Canada as well. As indicated by the map (Fig. 12) our collections were all made near the coasts of the Atlantic or Pacific Oceans, or the Gulf of Mexico.

#### Hosts

All active stages, except the male (which is undescribed), have been collected on birds of many species. However, we are including in the records one male, taken by itself, on a white-throated sparrow at Summerville, S. C., by William P. Wharton. We consider this specimen to be *Ixodes brunneus*, as it agrees well with the specific characters of the female.

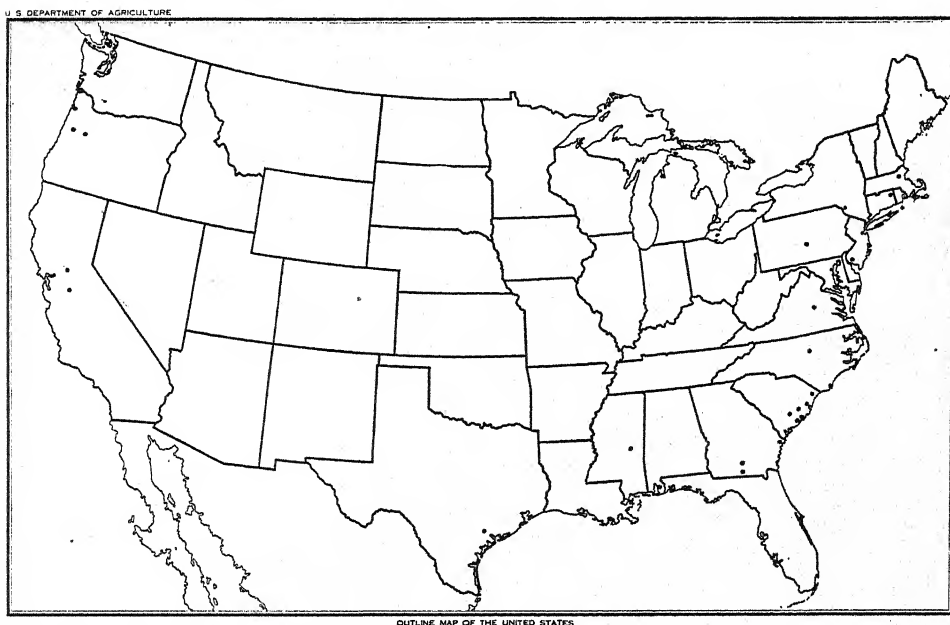


FIG. 12. Distribution of *Ixodes brunneus* in the United States, as indicated by collections of the Bureau of Entomology and Plant Quarantine.

Nuttall and Warburton (1911) summarized the collection records published up to that time, and listed several hosts not represented in the Bureau collections. Our collections, given in Table 11, contain specimens taken on birds every month except May and June. The fact that less bird banding and collecting are done during early summer probably accounts for the absence of records in those months.

#### *Ixodes cookei* Pack.

*Ixodes cookei* is not of great economic importance, as it is not known to transmit disease or to annoy man or other animals seriously. The long mouth parts, characteristic of all *Ixodes*, make this species especially troublesome when it does attach to man. The mouth parts are frequently broken off in the tissues of the host.

#### Distribution

*Ixodes cookei* is a widely distributed species (Fig. 13), but most of our collec-

tions have come from the eastern part of the United States. Eight of the 11 lots recorded here from man are from the New England States; 2 are from Pennsylvania, and 1 from Quebec.

### Hosts

Wild mammals are the chief hosts, although a number of adult ticks have been collected from dogs and several from man. All stages are found on animals through-

TABLE 12.—*Ixodes cookei*, *I. cookei* var. *rugosus*, *I. dentatus* and *I. diversifossus*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
<i>Ixodes cookei</i>					
Cat, civet .....	1	.....	.....	.....	1(1p)
Cat, domestic .....	2	.....	.....	.....	2(2p)
Cattle .....	3	.....	.....	1(1)	2(2pf)
Cougar, Oregon .....	1	.....	.....	.....	1(1)
Dog .....	29	1(1)	11(+)	.....	18(+ up)
Ferret .....	1	.....	1(3p)	.....	.....
Fox .....	3	.....	2(10sf)	.....	1(1p)
Fox, gray .....	9	5(58sf)	6(10uf)	.....	4(10sf)
Ground squirrel, brown	1	.....	.....	1(1)	1(1s)
Ground squirrel, striped .....	1	.....	1(11s)	.....	.....
Man .....	11	.....	5(+ up)	.....	8(+ sp)
Mink .....	7	2(+ up)	4(+ pf)	.....	5(+ sf)
Mouse, "field" .....	1	.....	.....	.....	1(+ s)
Mouse, northern golden	1	1(1p)	.....	.....	.....
Opossum .....	8	1(6f)	6(+ sp)	.....	1(1s)
Porcupine .....	2	.....	.....	.....	2(6pf)
Prairie dog .....	2	.....	2(5u)	.....	.....
Raccoon, eastern .....	3	1(13sp)	3(+ sp)	.....	1(1p)
Skunk .....	6	.....	4(51pf)	.....	4(9sp)
Skunk, Alleghenian spotted .....	2	.....	2(2p)	.....	1(3sp)
Skunk, Florida .....	6	1(15uf)	2(+ 150)	1(5)	5(15+sp)
Skunk, Rio Grande spotted .....	1	.....	1(10pf)	.....	1(1s)
Squirrel, fox .....	1	.....	1(+ s)	.....	.....
Squirrel, gray .....	2	.....	2(+ 10p)	1(1)	.....
Weasel .....	2	2(12sf)	5(14sp)	.....	4(5p)
Weasel, New York .....	2	.....	1(2p)	.....	1(1s)
Woodchuck .....	6	1(38sp)	5(1247sf)	.....	4(8sp)
Woodchuck, pallid yellow-bellied .....	1	.....	1(3p)	.....	.....
Woodchuck, southern .....	1	1(4)	1(2f)	.....	.....
Woodchuck, yellow-bellied .....	1	1(18sp)	.....	.....	1(2p)
<i>Ixodes cookei</i> var. <i>rugosus</i>					
Dog .....	10	2(2p)	6(29uf)	.....	5(10sp)
Ground squirrel, Columbian .....	1	.....	.....	.....	1(1p)
Weasel .....	1	.....	.....	.....	1(2p)
<i>Ixodes dentatus</i>					
Mouse, meadow .....	3	1(1)	2(3sp)	.....	.....
Muskrat .....	3	2(3pf)	.....	1(1)	.....
Quail, bobwhite .....	1	1(1p)	.....	.....	.....
Rabbit, cottontail (including New England cottontail and eastern cottontail) .....	143	89(6407uf)	89(1097uf)	41(311)	51(560uf)
Sheep .....	1	.....	1(1s)	.....	.....
Sparrow, song .....	1	1(1u)	.....	.....	.....
Sparrow, white-crowned .....	1	.....	1(1u)	.....	.....
Thrasher, brown .....	2	1(1f)	1(3s)	.....	.....
<i>Ixodes diversifossus</i>					
Chipmunk, Townsend .....	1	.....	.....	.....	1(1p)
Cow .....	1	.....	.....	.....	1(1s)
Hare, various species .....	2	.....	.....	.....	2(3pf)
Rabbit, cottontail .....	8	1(29pf)	1(1f)	3(1)	6(27uf)
Rabbit, jack (including black-tailed and Washington) .....	7	.....	1(1p)	3(18)	6(22sf)
Rabbit, Rocky Mountain cottontail .....	2	.....	1(2pf)	1(7)	2(26sf)
Sparrow, valley fox .....	1	.....	1(1s)	.....	.....
Wood rat .....	1	.....	.....	.....	1(1p)

out the year in the southern half of the country. We have specimens of nymphs taken in Rochester, N. Y., on December 21, 1917, and 2 females taken on weasel December 28, 1932, and 1 female on weasel, January 14, 1933, in Pennsylvania. In the Northern States, however, it is most abundant on hosts in midsummer.

Bureau of Entomology and Plant Quarantine records are given in Table 12.

*Ixodes cookei* var. *rugosus* Bishopp

*Ixodes cookei* var. *rugosus* is apparently of no economic significance although it may occur on dogs in moderate numbers in all motile stages.

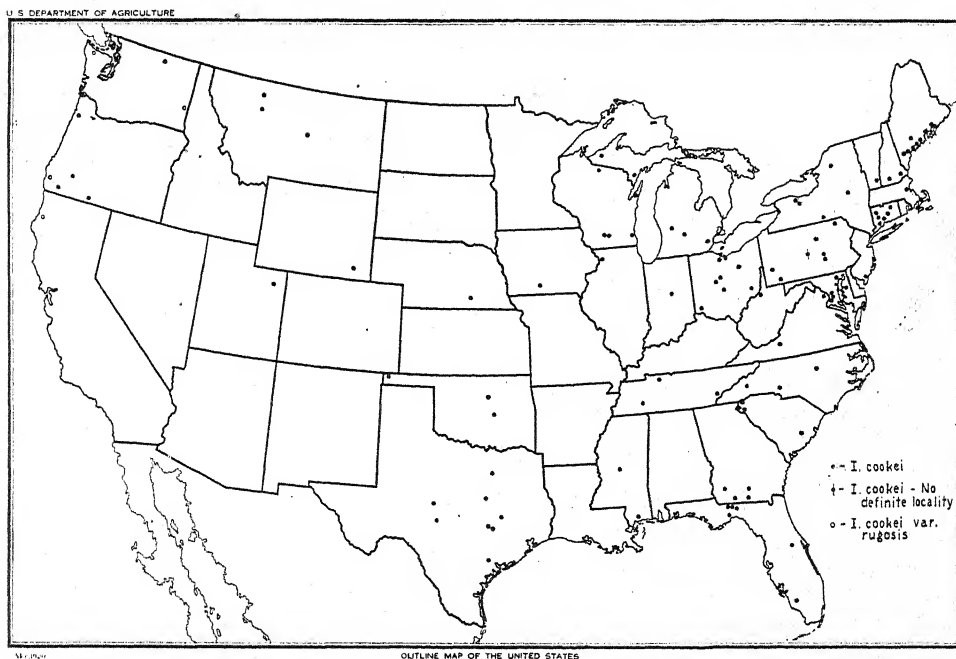


FIG. 13. Distribution of *Ixodes cookei* and *I. cookei* var. *rugosus* in the United States, as indicated by collections of the Bureau of Entomology and Plant Quarantine.

*Distribution and Hosts*

All the specimens (10 lots) in the collection of the Bureau of Entomology and Plant Quarantine are from western Washington and Oregon (see Fig. 13).

Larvae were collected in March, nymphs in March, May, September, November, and December, and females in March, May, July, September, and November.

The collection records of the Bureau of Entomology and Plant Quarantine are summarized as shown in Table 12.

*Ixodes dentatus* Neum.

The species *Ixodes dentatus* confines its attack rather closely to cottontail rabbits and appears to be of little economic importance, although it may serve as a carrier of tularemia among rabbits. It is frequently found on these animals in sufficient numbers to cause much irritation and loss of blood.

At present, there is some confusion as to the identity of *Ixodes dentatus* and *I. diversifossus*, as will be explained in the discussion of the latter species.



### Distribution

*Ixodes dentatus* was described from specimens in the Marx collection taken in Maryland from rabbit. It is particularly abundant along the coast from Maryland to Massachusetts (Fig. 14). Numerous collections made by C. N. Smith, M. M. Cole, and H. K. Gouck on Martha's Vineyard, Mass., show the species to be very plentiful there.

### Hosts

*Ixodes dentatus* is found almost exclusively on wild mammals. The rabbit is the type host, and it is certainly the preferred one. The writers, however, have 5

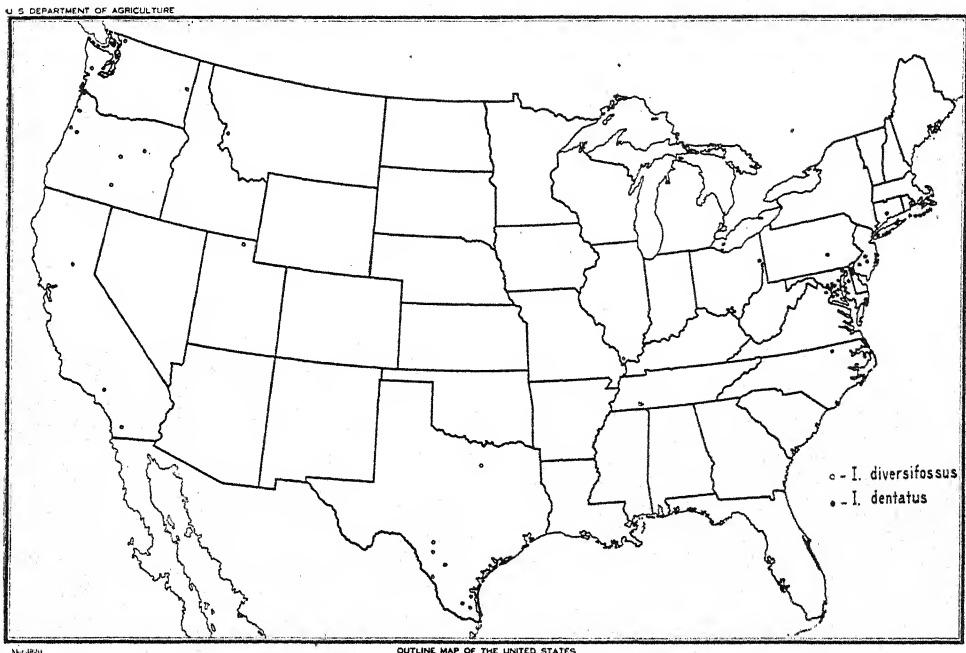


FIG. 14. Distribution of *Ixodes diversifossus* and *I. dentatus* in the United States, as indicated by collections of the Bureau of Entomology and Plant Quarantine.

lots of larvae and nymphs, totaling 7 specimens, which they consider to be this species, taken on birds. The Bureau collections given in Table 12 contain specimens taken on hosts every month of the year. Quite often all stages are found together on the same host. Males are much less numerous on hosts than are females, and they were collected only from April to July, inclusive. The maximum abundance was in April. The species is frequently found on cottontail rabbits in very large numbers. For instance, one rabbit collected by C. N. Smith at Edgartown, Mass., on May 5, 1940, carried 733 larvae, 205 nymphs, 55 males, and 91 females. One lot of 4 fully engorged larvae were collected in a grass clump on February 24, 1940, at Arlington, Va. Only one individual of the species, a slightly engorged nymph, was collected on a cloth drag.

### *Ixodes diversifossus* Neum.

The rabbit appears to be the preferred host of all stages of *Ixodes diversifossus*. At times this tick becomes sufficiently abundant on rabbits to cause some ill effects.

It is not infrequently associated with the rabbit tick (*Haemaphysalis leporispalustris*) on rabbits. It is rarely found on domestic animals, and it is therefore of little economic importance, although it may play a part in transmitting tularemia among rabbits.

#### Distribution

This is a North American species found mainly in the western half of the United States. It probably occurs in northern Mexico and southwestern Canada. The type specimens were from New Mexico. There has been some confusion involving this and related species, and this confusion is not yet entirely cleared. It appears that, in general, *Ixodes dentatus* is found in the East, and *I. diversifossus* in the western and southwestern part of the country. For the present purpose we are regarding the specimens from the West and Southwest as *I. diversifossus*. The accompanying map (Fig. 14) indicates the distribution of *diversifossus* and *dentatus*. Cooley and Kohls (1942) regard the form found in the Northwest as a species (*spinipalpis* Nuttall) distinct from *diversifossus* and *dentatus*.

#### Hosts

Host records obtained by the Bureau of Entomology and Plant Quarantine indicate that the species confines its attack almost wholly to mammals. We have only one specimen from a bird host. This is a slightly engorged nymph collected on a valley fox sparrow, March 5, 1930, at Netarts, Ore., by Alex and Rosaline Walker.

Specimens have been taken on hosts every month of the year except August. Larvae and nymphs are not commonly found on hosts, and the collections containing them were taken in the spring, fall, and winter. Bureau of Entomology and Plant Quarantine records are given in Table 12.

#### *Ixodes kingi* Bishopp

##### The Rotund Tick

The rotund tick is of no recognized economic importance except as a parasite of dogs and certain fur-bearing animals. However, like many other ticks it may at times be concerned with the transmission among animals of such diseases as tularemia and Rocky Mountain spotted fever.

#### Distribution

The type locality of *Ixodes kingi* is Meeteetse, Wyo. Some Bureau records on the distribution of this species have been published by Bishopp (1911a). These and more recent records are indicated on the accompanying map (Fig. 16). It will be noted that the species is found mainly in the western half of the United States. It has been recorded from Medicine Hat, Alberta, by Hearle (1938), and with little doubt it occurs also in northern Mexico.

#### Hosts

The type host is badger, and this appears to be a preferred host since next to the dog the largest number of collections and of specimens recorded in our files are from that host. The species is seldom found in any considerable numbers on any host.

All stages were taken on hosts practically throughout the year, although they appear to be most abundant during spring and early summer. The Bureau collections are shown by hosts in Table 13.

TABLE 13.—*Ixodes kingi*, *I. muris*, and *I. ricinus californicus*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
<i>Ixodes kingi</i>					
Badger .....	6	.....	1(4p)	4(10)	6(54sp)
Dog .....	20	.....	1(1p)	4(10)	20(58uf)
Ferret, black-footed ..	1	.....	.....	.....	1(1s)
Fox, red .....	1	.....	.....	.....	1(1p)
Ground squirrel, Co-	2	1(6u)	1(4s)	.....	.....
lumbian .....	2	.....	.....	.....	2(4p)
Mink .....	4	.....	.....	1(1)	4(4p)
Pocket gopher ( <i>Thomo-</i>	1	.....	.....	.....	1(1p)
<i>mys</i> sp.) .....	4	.....	1(3sp)	1(1)	2(2u)
Pocket mouse, Oregon.	1	.....	.....	.....	Several
Prairie dog .....	1	.....	1(1p)	.....	.....
Rabbit, cottontail .....	1	1(2f)	.....	1(1)	.....
Raccoon .....	1	.....	1(25up)	.....	.....
Rat, kangaroo .....	1	.....	.....	.....	.....
Skunk .....	2*	.....	1(4p)	.....	.....
Skunk ( <i>Comptaxis lemo-</i>	3	.....	2(5sf)	.....	3(15sf)
<i>natus texanus</i> ) .....	1	.....	.....	.....	1(3p)
Skunk ( <i>Mesomelas lon-</i>	1	.....	.....	.....	1(1p)
<i>gata</i> ) .....	2	.....	.....	.....	1(1s)
Skunk ( <i>M. mesomelas</i>	1	.....	.....	.....	.....
<i>varians</i> ) .....	1	.....	.....	.....	.....
Wolf .....	2	.....	1(3p)	.....	.....
Woodchuck .....	3	.....	2(2s)	.....	1(5p)
Woodchuck, pallid yel-	1	1(3sp)	.....	.....	.....
low-bellied .....					
Wood rat .....					
<i>Ixodes muris</i>					
Dog .....	2†	.....	1(1)	.....	1(1p)
Man .....	1	.....	2(3up)	.....	.....
Mouse, jumping .....	3	.....	.....	.....	.....
Mouse, meadow (includ-	13	5(8u)	6(18u)	1(1)	6(23up)
ing Pennsylvania	4	1(1u)	3(5)	.....	2(3)
meadow mouse) .....	3	.....	3(4f)	.....	1(1f)
Mouse, white-footed ..	6	1(3f)	1(2)	2(6)	5(11uf)
Muskrat .....	1	1(2u)	.....	.....	.....
Rabbit, cottontail .....	3	2(2f)	.....	.....	.....
Rat .....	1	1(5)	1(2)	.....	.....
Rat, Norway .....	1	.....	.....	.....	1(1)
Shrew, long-tailed .....					
Shrew, short-tailed .....					
<i>Ixodes ricinus californicus</i>					
Bobcat .....	4	.....	.....	3(9)	4(6uf)
Burro .....	1	.....	.....	.....	1(2sp)
Cat, domestic .....	1	.....	.....	.....	1(1p)
Cattle .....	14	.....	.....	6(14)	14(72uf)
Cougar .....	3	.....	.....	1(8)	3(13sp)
Coyote .....	5	.....	.....	2(4)	4(12uf)
Deer .....	3	.....	.....	3(21)	3(24up)
Deer, black-tailed .....	2	.....	.....	2(18)	2(7sp)
Dog .....	34	.....	1(5pf)	16(34)	34(123up)
Ground squirrel .....	1	.....	1(1s)	.....	.....
Grouse .....	1	1(8f)	1(12sp)	.....	.....
Horse .....	18	.....	1(1)	6(6)	15(+ 26sp)
Lizard ( <i>Sceloporus o.</i>	2	.....	2(6sp)	.....	.....
<i>occidentalis</i> ) .....	22	.....	.....	8(14)	26(36up)
Man .....	1	.....	1(1u)	.....	.....
Mouse, meadow .....	1‡	.....	.....	.....	.....
Mouse, southern para-	1	.....	.....	.....	.....
sitic .....	1	1(7sf)	.....	.....	.....
Mouse, spiny pocket ..	2	.....	.....	1(1)	2(3s)
Mule .....	1	1(1p)	.....	.....	.....
Owl, burrowing .....	1	.....	.....	1(2)	.....
Rabbit .....	1	.....	.....	.....	.....
Rabbit, Colorado desert	1	.....	.....	1	.....
jack .....	1	.....	.....	.....	1(1s)
Rabbit, cottontail .....	3	1(1f)	.....	.....	2(2sp)
Rabbit, jack .....	2§	.....	.....	1(1)	1(2sp)
Wolf, timber .....	1	.....	1(1s)	.....	.....
Wood rat, large-eared ..					

\* One lot included "2 ticks."

† One lot included "6 ticks."

‡ One tick. § Eight hides examined.

*Ixodes marxi* Banks

## Marx Tick

The Marx tick is of no recognized economic importance, and it is certainly a relatively rare species. The collections of the Division of Insects Affecting Man and Animals of the Bureau of Entomology and Plant Quarantine contain only eight lots; and eight (females) was the maximum number taken on any one host (gray squirrel, by E. B. Marshall). The male is unknown and apparently does not attach to hosts.

*Distribution and Hosts*

In describing the species, Banks (1908) records specimens taken on red squirrel in Washington, D. C., Salineville and Wauseon, Ohio, Ithaca, N. Y., Portland, Mich., and Guelph, Ontario, and on fox in Denver, Colo.

The records on some of the above-mentioned collections and subsequent ones (Bureau of Entomology and Plant Quarantine data) are: Gray squirrel: Laurel, Md., September 15, 1932, numph (1 slightly engorged), females (2 partly engorged); Langley Park, Md., January 15, 1937, females (8 partly engorged); Nile Township, Scioto County, Ohio, October 3, 1936, female (1 partly engorged); Dauphin County, Pa., March 27, 1940, nymph (1 slightly engorged), female (1 slightly engorged). Red squirrel: Webster, N. Y., October 1915, female (1 unengorged); Ashtabula, Ohio, September 16, 1937, females (5 partly engorged); Faulkland, Del., May 20, 1941, female (1 slightly engorged); Toronto, Canada, August 26, 1923, female (1 slightly engorged).

Hearle (1938) states that specimens that agree very closely with the description of this species have been found in the Kamloops and Nicola districts and the North Thompson Valley in British Columbia. These were taken almost entirely on the pine squirrel.

*Ixodes muris* Bishopp & Smith

## The Mouse Tick

The mouse tick is of no recognized economic importance. It is possible that it may play a part in the transmission of disease among rodents. It appears never to be very abundant, as indicated in the statement by Bishopp and Smith (1937) that on 103 rodents were found 1 larva, 25 nymphs, 1 male, and 24 females.

*Distribution*

This species has been collected only on the southern side of Cape Cod and the adjacent islands of Martha's Vineyard and Nantucket, with the exception of a single lot from Melvin Village, N. H.

*Hosts*

This tick was taken on hosts during the period April to October only.

The predominant hosts are mice, but occasionally this species attaches to other mammals as shown by the Bureau of Entomology and Plant Quarantine records in Table 13.

*Ixodes ricinus californicus* Banks

## The California Black-Legged Tick

The California variety of the European *Ixodes ricinus* is of distinct importance as a pest of man, domestic animals, and deer along the West Coast from Mexico into



British Columbia. In some localities it is the dominant wood tick, and it may well be concerned in the transmission of rickettsial or other diseases of man and animals.

Cooley and Kohls (1943) state that Banks' type of *Ixodes californicus* is not specifically identical with the form that has been generally referred to as *I. californicus* or *I. ricinus californicus* in the literature (and to which we refer here). They propose a new name, *Ixodes pacificus*, for this form.

#### Distribution

This form is common in the Pacific States, mainly west of the Cascade Range (Fig. 15). Its range extends into British Columbia and southward into Lower California. One of the Utah records shown on the map represents a female tick that

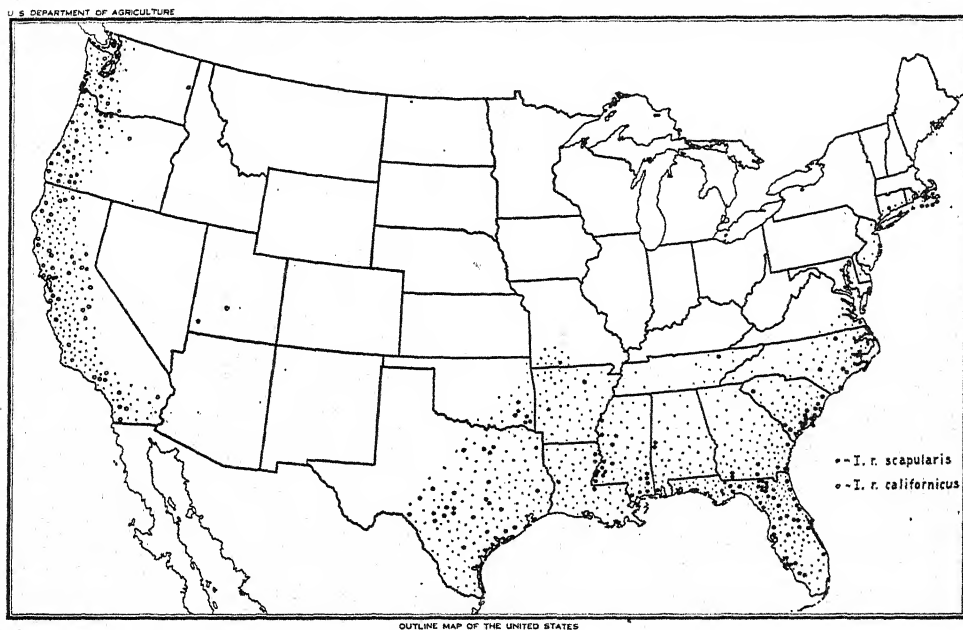


FIG. 15. Distribution of *Ixodes ricinus scapularis* and *I. r. californicus* in the United States. Large dots and circles indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

attached, on October 20, 1938, to the armpit of a hunter, who may have picked it up from a deer which he had killed. Gerald Thorne who collected this specimen writes: "No additional collections of *Ixodes ricinus californicus* have been made since the first one in 1938. We hunted in the same area (Birch Creek, southwest of Circleville, Utah) in 1939, 1940, and 1941, and I examined deer and porcupines for additional specimens but without success. Milo H. Denning of the Forest Service recalled that about 1930 he found a similar tick 20 miles southwest of Circleville attached at the belt line."

The other record on the map refers to two female ticks submitted by Dr. Don Rees. These attached to a man on a deer hunt near Pinto, Utah, October 20, 1938. These may well have come from a deer. Both the immature and adult stages are most abundant in winter, spring, and early summer. None of the collections of the Bureau was made in August and September, and a few in July and October.

### Hosts

The adults of the California black-legged tick have a rather broad host range among mammals. In addition to attacking mammals, the immature stages attach to cold-blooded animals, such as lizards, and occasionally are found on birds.

Bureau host records are given in Table 13.

### *Ixodes ricinus scapularis* Say

#### The Black-Legged Tick

Although not known to be concerned in the transmission of any disease, the black-legged tick is of considerable importance as a parasite of many animals, including man. Its long mouth parts inflict a noticeable wound and are often broken off in the tissues when the tick is removed. This form is generally regarded as a variety of the European *Ixodes ricinus*. However Cooley (1944) in an article received as this goes to press, apparently considers *scapularis* a distinct species. He also described in this paper a new species (*Ixodes ozarkus*) from dogs in Arkansas. We think we have included among our records of *I. r. scapularis*, specimens of this form (*I. ozarkus*) which is very closely related to *scapularis*.

### Distribution

The black-legged tick is found from southern Massachusetts southward to Florida, and from Indiana and Iowa south to Louisiana and Texas (Fig. 15). A few specimens have been collected in Ontario, Canada (Nuttall, 1911, p. 158). The species also extends into Mexico, and Banks considers as this form some specimens from Costa Rica described by Neumann as *Ixodes affinis*. This tick has been collected by workers of the Bureau of Entomology and Plant Quarantine in every month of the year, but in the South it is most abundant during the fall and spring. During the summer the immature stages are found on hosts more commonly than are the adults.

### Hosts

The black-legged tick is a very general feeder on mammals. Cattle, deer, dogs, and hogs are often rather heavily infested. Hooker, Bishopp, and Wood (1912) report the immature stages as collected on quail, blue jay, and thrush, and we here add the towhee. The immature stages attack lizards also.

Collections of the Bureau of Entomology and Plant Quarantine indicate that a considerable number of these ticks are recovered on clothing or on cloths dragged over the vegetation. Other records are shown in Table 14.

### *Ixodes sculptus* Neum.

*Ixodes sculptus* is parasitic on mammals, particularly on wild species. It is not recognized as being of economic importance, although it may play a part in the transmission of such diseases as tularemia or Rocky Mountain spotted fever. The males are rarely found on hosts. As pointed out by Hixson (1932), the habitat of the species except while on animals is commonly in the burrows of rodents. The engorged ticks leave the hosts at night and burrow into the litter, where copulation, molting, and oviposition take place.

Considerable confusion exists regarding the identity of this species because of a rather close resemblance to certain other species.

TABLE 14.—*Ixodes ricinus scapularis*, *I. sculptus*, and *I. texanus*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
<i>Ixodes ricinus scapularis</i>					
Bobcat, Florida .....	3	.....	1(1f)	3(23)	3(25sp)
Cardinal .....	1	.....	.....	1(1)	4(6uf)
Cat, domestic .....	4	.....	.....	29(+76)	69(+235uf)
Cattle .....	70	.....	.....	1(41)	1(31sp)
Deer, Mexican (hide) ..	1	.....	.....	54(1197)	66(+1194uf)
Deer, white-tailed .....	65	1(25)	2(10)	30(+317)	60(+1220)
Dog .....	65	1(8)	3(13)	2(5)	3(26up)
Fox .....	3	.....	.....	.....	2(+19)
Goat .....	2	.....	.....	2(3)	10(68uf)
Hog .....	11	.....	.....	4(14)	14(57uf)
Horse .....	14	.....	.....	.....	.....
Lizard, red-headed .....	1	1(1)	1(1p)	.....	.....
Lynx ( <i>Lynx</i> spp.) .....	4	.....	.....	1(3)	4(23sf)
Man .....	21	.....	4(4us)	8(19)	14(29up)
Mouse, cotton .....	3	2(2s)	1(1s)	.....	.....
Mouse, meadow and white-footed .....	3	2(27f)	3(12uf)	.....	.....
Mouse, northern white-footed .....	1	.....	.....	1	.....
Mouse, old-field .....	1	1(1p)	.....	.....	.....
Mule .....	2	.....	.....	.....	2(5p)
Opossum .....	9	.....	.....	3(12)	9(64uf)
Raccoon .....	10	.....	.....	4(7)	9(16up)
Rat, Norway .....	1	1(1)	.....	.....	.....
Rat, rice swamp .....	1	1(3p)	.....	.....	.....
Sheep .....	4	.....	.....	3(14)	4(40uf)
Skink .....	1	.....	1(1)	.....	.....
Skink, red-headed .....	1	.....	1(5up)	.....	.....
Squirrel .....	2	.....	1(1)	.....	1(1s)
Towhee .....	2	1(1u)	2(11uf)	.....	.....
Wolf .....	1	.....	.....	.....	1(2f)
<i>Ixodes sculptus</i>					
Cat, civet .....	2	.....	2(11sp)	.....	2(6p)
Cat, domestic .....	1	.....	.....	.....	1(1p)
Fox, red .....	1	.....	.....	.....	1(1s)
Goat, domestic .....	1	.....	.....	.....	1(2sp)
Ground squirrel, Belding .....	1	.....	.....	.....	1(1)
Ground squirrel ( <i>Citellus</i> sp.) .....	1	.....	.....	.....	1(2us)
Ground squirrel, Franklin .....	1	.....	.....	.....	1(5sp)
Ground squirrel, striped .....	24	5(+18up)	15(48uf)	4(5)	17(30up)
Man .....	1	.....	1(2p)	.....	1(1p)
Mole, Missouri Valley ..	1	.....	.....	.....	.....
Mouse, redwood white-footed .....	1*	.....	.....	.....	.....
Pocket gopher, brown ..	2	1(90sf)	1(12p)	.....	2(18p)
Pocket gopher ( <i>Geomys</i> spp.) .....	3	.....	.....	1(1)	3(8sp)
Pocket gopher, Illinois ..	2	.....	.....	1(1)	1(17up)
Pocket gopher, prairie ..	1	.....	.....	.....	1(4p)
Pocket gopher, Shaw .....	1	.....	.....	.....	1(4s)
Prairie dog .....	1	.....	.....	.....	1(2us)
Rabbit .....	1	.....	.....	.....	1(1p)
Raccoon .....	1	.....	.....	.....	1(1p)
Skunk .....	2	.....	2(4sp)	.....	2(3p)
Skunk, "two-lined" .....	1	.....	.....	.....	1(1s)
Weasel .....	1	.....	1(1p)	.....	1(1p)
Wood rat .....	1	.....	1(2p)	.....	1(3p)
<i>Ixodes texanus</i>					
Bobcat .....	1	.....	2(3sf)	.....	1(1f)
Marten .....	5	.....	15(39sf)	3(4)	5(15pf)
Raccoon .....	30	4(12sf)	1(1p)	.....	23(66sf)
Squirrel .....	2	.....	4(23sp)	.....	2(5p)
Squirrel, pine .....	5	1(1s)	.....	.....	3(27sp)
Weasel .....	3	1(158pf)	2(7pf)	.....	3(4p)

\* "1 tick."

## Distribution

*Ixodes sculptus* is rather widely distributed from Illinois, Michigan, and Louisiana westward, but apparently it is not abundant anywhere. The type locality is the Santa Cruz Mountains in California. It has been recorded from Canada, and it may possibly occur in northern Mexico.

### Hosts

Burrowing rodents appear to be the preferred hosts. Possibly the predatory habit of some of the larger mammals, such as the skunk and weasel, upon which the tick has been found, accounts for their infestation.

We have but a single record of the occurrence of this species on man, and there is some doubt as to the identity of the specimens (2 nymphs) in this case. This collection was made by O. G. Babcock at Sonora, Tex. on March 30, 1921. Our records show the tick to have been collected on animals in every month of the year. The habit of the tick of spending its non-parasitic life in the burrows of animals would give it protection against unfavorable weather and favor its year-round activity.

Bureau of Entomology and Plant Quarantine host records are given in Table 14.

### *Ixodes texanus* Banks

*Ixodes texanus* is of no apparent economic importance, as it has been collected only on wild mammals, and even then not in great numbers.

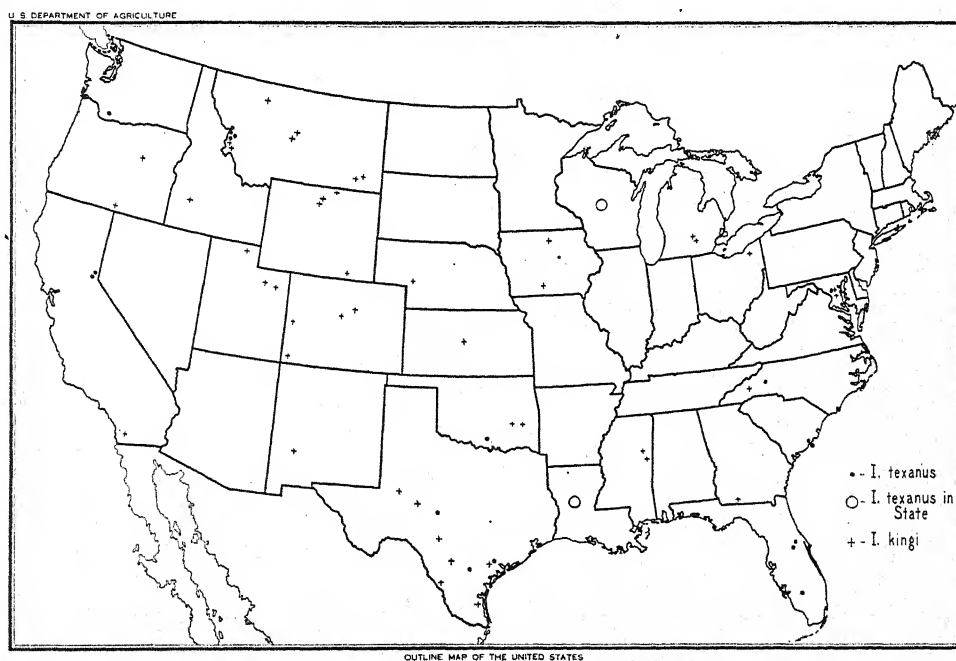


FIG. 16. Distribution of *Ixodes texanus* and *I. kingi* in the United States, as indicated by collections of the Bureau of Entomology and Plant Quarantine.

### Distribution

The type locality of this tick is Live Oak County, Texas. The species is widely distributed in the United States and southern Canada. No doubt it occurs in northern Mexico also. The accompanying map (Fig. 16) indicates its distribution in the United States.

### Hosts

The type host is raccoon (*Procyon lotor*). This animal appears to be one of the preferred hosts. All our collections have been from wild mammals, but Eddy and

Joyce (1942) report 1 female from a dog in Iowa. This species is found in all active stages on mammals throughout the year. We have specimens collected in every month except October. Most of the collections were taken in the winter, but this is probably because most fur-bearing animals are caught at this time of year.

Males of this species are rarely taken on hosts. Four males in the collections were from three raccoons caught in March and May. On two of these animals the males were associated with females. In 1912 the senior author pointed out that the nymphs collected up to that time were taken only during the period July to November. More recent collections show them, and larvae also, to be on hosts during winter and spring as well. In one case, the comparatively large number of 158 larvae, 4 nymphs, and 1 female were taken on a weasel at Lake Tahoe, Calif. on January 30, 1917. The Bureau host records are given in Table 14.

#### *Ornithodoros megnini* Meig.

##### The Ear Tick

The ear tick, or, as it is sometimes aptly called, "the spinose ear tick," is a native of the Americas. It claims attention as a parasite of all classes of domestic animals, certain of the larger wild mammals, and man.

It is not known to transmit any disease, but its presence is highly irritating and often painful. Since this tick attaches deep in the external ear it is well protected from scratching and also from acaricides applied to livestock as a dip. The irritation produced often leads to attack by screwworms, which sometimes results in disfigurement or death.

##### Distribution

In its original range, the ear tick was probably confined to the semi-arid and arid Southwestern States and Mexico. Its habit of remaining in the deeper portions of the ears of animals in the larval and nymphal stages for considerable periods (up to 7 months) affords an opportunity for it to be transported long distances with its hosts. Also, the facts that the adults take no food, that they often hide in protected places, and that they continue to deposit eggs over periods of several months permit this pest to spread in stock cars and other vehicles used in hauling livestock.

It appears that the ear tick may survive and breed temporarily in the more humid portions of the Southern States and in the relatively dry and cold northern Rocky Mountain States. The tick was reported in considerable numbers in the ears of horses on a farm near Orlando, Fla. in 1912. Specimens were collected by W. W. Yothers on January 17, and the senior author visited the locality on March 31 and examined four of the horses, one of which had two nymphs in one ear. These horses had been shipped from Alamogordo, N. Mex., during the previous year. Inquiries indicated that although horses were frequently shipped there from normally infested areas of the United States the species had not become established.

In connection with the distribution of *Ornithodoros megnini* by shipments of livestock, it is interesting to note that this species was accidentally introduced into the Karoo and western South Africa, presumably in shipments of horses or mules from the southwestern part of the United States. Bedford (1934, p. 79) states that "It appears to have been observed by Manley in the Cape Colony as far back as 1898." He gives the distribution as "throughout the arid districts of Cape Province and



Orange Free State," also parts of Natal and the Transvaal as far north as the Pretoria District. Bedford (1913) first reported this tick from Africa, and gave Vryburg, Bechuanaland, Fauresmith, Orange Free State, Sudan, the United States, and Mexico as its habitats.

According to a report by J. H. Beaumont (1943), this tick is present in Hawaii. He states that 45 per cent of local cattle examined were infested with from 1 to 65 ticks in each ear.

It is unlikely that *Ornithodoros megnini* will ever become permanently established or assume much importance as a livestock pest in States to the east of Texas and to the north of Oklahoma, New Mexico, Arizona, and California, with the possible exception of certain parts of Southern Colorado, Utah, and Nevada. Cooley and Kohls (1944b), however, record an apparent hold-over for 4 years in Montana,

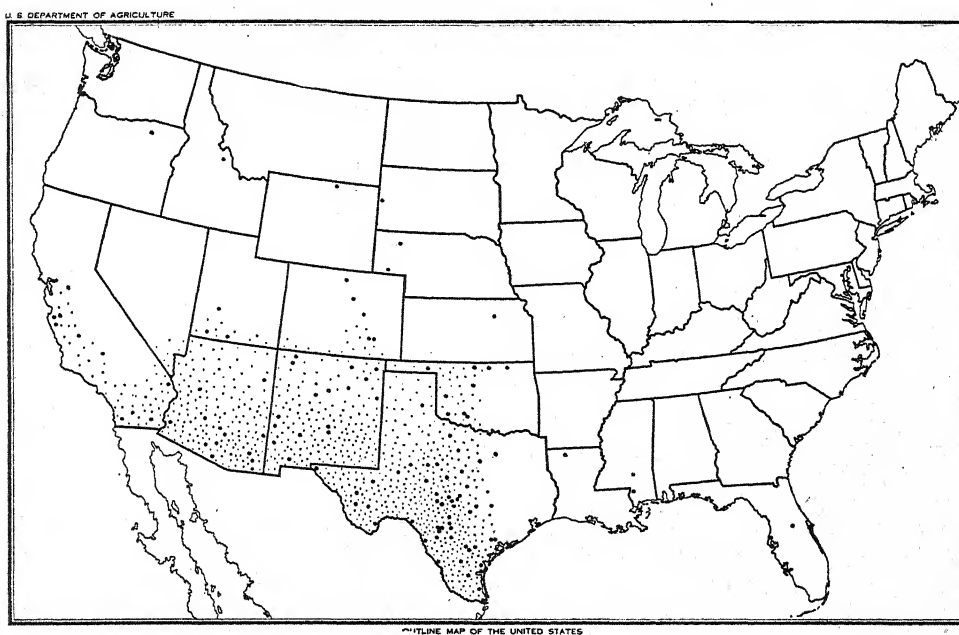


FIG. 17. Distribution of *Ornithodoros megnini* in the United States. Large dots indicate localities from which specimens are herein reported. Small dots indicate probable distribution of species.

from 1912 to 1916. This tick (4 well-engorged nymphs) was collected by J. D. Gregson in December 1943 in the ears of a mountain goat at Bryant Creek, British Columbia. The collection of a nymph on a cat in the same vicinity (Ewings Landing, British Columbia, October 6, 1941) suggests that the species may be established there, at least temporarily. Likewise it will probably never become a serious pest in areas of considerable rainfall. The eastern boundary of the tick's occurrence in numbers is approximately the 97th meridian, as shown on the accompanying map (Fig. 17).

#### Hosts

As has been mentioned, the ear tick attacks all kinds of large mammals. It is particularly troublesome as a pest of horses, cattle, sheep, and dogs. It is to be found on hosts throughout the year, but its injurious effects are specially noticeable during

winter and spring, particularly following long periods of drought when both the range and the livestock are poor. Theiler (1921) lists the ostrich as a host in addition to the common domestic animals. The six ticks reported on man were all from deep in the external ear. One nymph was said to have been causing pain for 3 months in the ear of a 3-year-old boy.

Table 15 gives the host records in the collection of the Bureau of Entomology and Plant Quarantine.

TABLE 15.—*Ornithodoros megnini*: Host records of the Bureau of Entomology and Plant Quarantine

Host	Lots	Larvae	Nymphs	Males	Females
Ass .....	6	3(+)	6(+)	.....	.....
Cat .....	12	1(1f)	11(+ uf)	.....	.....
Cattle .....	114	14(+ uf)	110(156uf)	.....	.....
Cattle flytrap .....	10*	.....	.....	.....	.....
Coyote .....	3	.....	3(19sp)	.....	.....
Deer .....	1	.....	1(6sp)	.....	.....
Deer, white-tailed .....	3	.....	3(5pf)	.....	.....
Dog .....	21	1(2)	21(53+ uf)	.....	.....
Elk .....	2	.....	1(4f)	.....	.....
Goat, Angora .....	1	.....	1(6sp)	.....	.....
Goat, mountain .....	1	.....	1(1s)	.....	.....
Hog .....	2	1(+)	2(+ 4pf)	.....	.....
Horse .....	20	.....	24(+ 97)	.....	.....
Man .....	6	1(1f)	5(6sp)	.....	.....
Mule .....	1	1(+)	1(+)	.....	.....
Rabbit, cottontail .....	1	.....	1(1u)	.....	.....
Rabbit, jack .....	1	.....	1(2p)	.....	.....
Sheep .....	7	.....	7(+ 73uf)	.....	.....

\* Sixteen males and females.

### *Rhipicephalus sanguineus* Latr.

#### The Brown Dog Tick

The brown dog tick is a first magnitude pest of dogs. Not only does it cause dogs much discomfort and loss of blood from its irritating bites, but it carries malignant jaundice (*Piroplasma canis*) of dogs in the Mediterranean region and in other parts of the world. This disease has been found in Florida, but it is not known to occur in other parts of the United States. The brown dog tick has been shown capable of carrying Spanish relapsing fever and typhus fever in the Mediterranean area. It has also been found able to transmit, experimentally, several other diseases including *Haematozoon canis*, *Trypanosoma cruzi*, *T. christophersi*, Rocky Mountain spotted fever, and anaplasmosis of cattle and dogs.

The brown dog tick is an annoying household pest. When dogs are kept in living quarters this tick often multiplies there, and all active stages are found crawling about the curtains, walls and furniture. Fortunately it does not readily attach to man, and it is not known to carry any disease of man in the United States. Its eradication is difficult.

#### Distribution

The brown dog tick is widely distributed throughout the tropical and temperate regions of the world. It has been collected on every continent. In the United States it was first found in southern Texas. Hooker, Bishopp, and Wood (1912) reported its occurrence as far north as Jackson and Hays Counties, and as far west as Del Rio, Val Verde County, Tex. Since then it has spread to practically all parts of the country (Fig. 18), but it is still more generally distributed in the Southwest. In the North it is more or less confined to the larger cities where dogs are con-

centrated. The transportation of dogs and their contact in boarding kennels give opportunities for its rapid dissemination. Although this tick occurs normally in the warmer parts of the world, the heated buildings in more northern regions are favorable for its overwintering and continuous development, therefore it is there confined chiefly to urban areas and to buildings. In those parts of the Southwest and in Florida where the climate is favorable it is found almost everywhere—out of doors as well as in buildings.

### Hosts

In the United States the dog is almost the exclusive host of the brown dog tick. In other parts of the world it is more general in its feeding habits, for instance it has been reported from dromedary, from fox and other canines, and from birds,

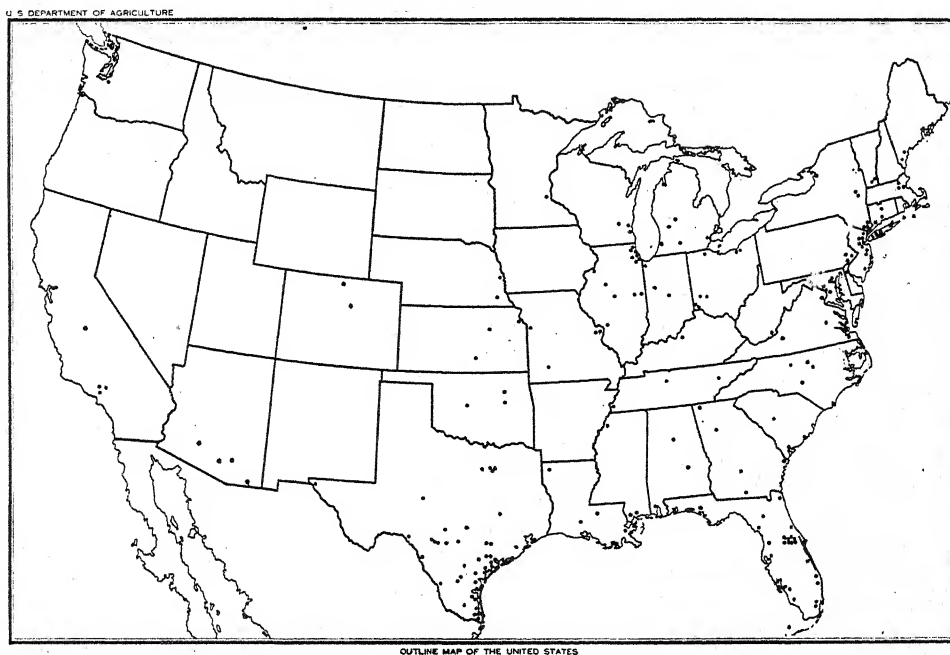


FIG. 18. Distribution of *Rhipicephalus sanguineus* in the United States, as indicated by collections of the Bureau of Entomology and Plant Quarantine.

buffalo, cat, goat, hare, horse, ox, and sheep. We have few United States records from hosts other than the dog, as indicated below. McIntosh (1931) states that the Zoological Division of the Bureau of Animal Industry has one record from a cow in West Palm Beach, Fla.

The Bureau of Entomology and Plant Quarantine collection contains records of one lot of 5 partly engorged females from ass; 408 lots from dog, consisting of numerous specimens of larvae, nymphs, and adults, in all stages of engorgement; 3 lots from rabbit, consisting of 2 males, 3 females, and 5 listed as "ticks." Two lots were sent in by correspondents with the report that they were taken from man; these consisted of 4 fully engorged larvae, 6 unengorged to fully engorged nymphs, 3 males, and 8 unengorged to fully engorged females. We consider these records from man doubtful.

## COMMON AND SCIENTIFIC NAMES OF HOSTS

<i>Common Name</i>	<i>Scientific Name</i>
Antelope, American pronghorn . . . .	<i>Antilocapra a. americana</i>
Badger . . . . .	<i>Taxidea taxus</i>
Blackbird, Brewer's . . . . .	<i>Euphagus cyanocephalus</i>
Blackbird, red-winged . . . . .	<i>Agelaius phoeniceus</i>
Bluebird, western . . . . .	<i>Sialia mexicana occidentalis</i>
Boa, Mexican . . . . .	
Bobcat . . . . .	<i>Lynx rufus</i>
Bobcat, Florida . . . . .	<i>Lynx rufus floridanus</i>
Bunting, painted . . . . .	<i>Passerina ciris</i>
Caribou . . . . .	<i>Rangifer</i> sp.
Catbird . . . . .	<i>Dumetella carolinensis</i>
Chaparral cock . . . . .	<i>Geococcyx californianus</i>
Chickaree, Douglas . . . . .	<i>Sciurus d. douglasii</i>
Civet cat . . . . .	<i>Bassariscus astutus</i>
Cony (see Pika) . . . . .	
Cowbird . . . . .	<i>Molothrus ater</i>
Chipmunk, buff-bellied . . . . .	<i>Eutamias amoenus luteiventris</i>
Chipmunk, Colorado . . . . .	<i>Eutamias q. quadrivittatus</i>
Chipmunk, Merriam . . . . .	<i>Eutamias merriami</i>
Chipmunk, painted . . . . .	<i>Eutamias minimus pictus</i>
Chipmunk, Townsend . . . . .	<i>Eutamias t. townsendii</i>
Coyote, Great Basin . . . . .	<i>Canis lestes</i>
Crow, southern . . . . .	<i>Corvus brachyrhynchos paulus</i>
Ctenosaura, spiny . . . . .	<i>Ctenosaura acanthura</i>
Deer, black-tailed . . . . .	<i>Odocoileus columbianus</i> subsp.
Deer, mule . . . . .	<i>Odocoileus hemionus</i> subsp.
Deer, Rocky Mountain . . . . .	<i>Odocoileus h. hemionus</i>
Deer, Virginia . . . . .	<i>Odocoileus v. virginianus</i>
Deer, white-tailed . . . . .	<i>Odocoileus virginianus</i> subsp.
Elk (wapiti), western . . . . .	<i>Cervus canadensis occidentalis</i>
Ferret, blackfooted . . . . .	<i>Mustela nigripes</i>
Finch, California purple . . . . .	<i>Carpodacus purpureus californicus</i>
Fox, Florida gray . . . . .	<i>Urocyon cinereoargenteus floridanus</i>
Fox, gray . . . . .	<i>Urocyon cinereoargenteus</i>
Fox, red . . . . .	<i>Vulpes</i> sp.
Goat, mountain . . . . .	<i>Oreamnos americanus</i>
Grackle, purple . . . . .	<i>Quiscalus q. quiscula</i>
Ground squirrel . . . . .	<i>Citellus</i> spp.
Ground squirrel, Belding . . . . .	<i>Citellus beldingi</i>
Ground squirrel, California or Beechey . . . . .	<i>Otospermophilus grammurus beecheyi</i>
Ground squirrel, Columbian . . . . .	<i>Citellus c. columbianus</i>
Ground squirrel, Douglas . . . . .	<i>Otospermophilus grammurus douglasii</i>
Ground squirrel, Franklin . . . . .	<i>Citellus franklini</i>
Ground squirrel, gilded . . . . .	<i>Callispermophilus chrysodeirus</i>

Common Name	Scientific Name
Ground squirrel, little gray	<i>Citellus m. mollis</i>
Ground squirrel, Montana mantled	<i>Callospermophilus lateralis cinerascens</i>
Ground squirrel, Oregon	<i>Citellus oregonus</i>
Ground squirrel, striped	<i>Citellus tridecemlineatus</i>
Ground squirrel, Wyoming	<i>Citellus elegans</i>
Grouse, Oregon ruffed	<i>Bonasa umbellus sabini</i>
Grouse, ruffed	<i>Bonasa umbellus</i>
Hawk, marsh	<i>Circus hudsonius</i>
Hawk, sparrow	<i>Falco sparverius</i>
Hawk, Swainson's	<i>Buteo swainsoni</i>
Heron, little green	<i>Butorides v. virescens</i>
Iguana, ctenosaura	<i>Ctenosaura multispinosus</i>
Jay, coast	<i>Cyanocitta stelleri carbonacea</i>
Junco, Shufeldt's	<i>Junco hyemalis connectens</i>
Killdeer	<i>Oxyechus v. vociferus</i>
Leopard-cat	<i>Felis pardalis griffithii</i>
Lion, Mexican	<i>Felis hipposlestes aztecus</i>
Lion, mountain	<i>Felis concolor</i>
Lizard	<i>Sceloporus undulatus</i>
Lizard	<i>Sceloporus o. occidentalis</i>
Marten	<i>Martes sp.</i>
Meadowlark, southern	<i>Sturnella magna argutula</i>
Mink, Florida	<i>Mustela vison lutensis</i>
Mockingbird	<i>Mimus polyglottos</i>
Mole, common	<i>Scalopus aquaticus</i>
Mole, Missouri Valley	<i>Scalopus aquaticus machrinoides</i>
Mole, prairie	<i>Scalopus aquaticus machrinus</i>
Moose	<i>Alces americana</i>
Mouse, Baird	<i>Perognathus f. flavus</i>
Mouse, Cary	<i>Microtus montanus caryi</i>
Mouse, cotton	<i>Peromyscus g. gossypinus</i>
Mouse, Gambel white-footed	<i>Peromyscus maniculatus gambeli</i>
Mouse, house	<i>Mus m. musculus</i>
Mouse, meadow	<i>Microtus sp.</i>
Mouse, northern golden	<i>Peromyscus n. nuttalli</i>
Mouse, northern white-footed	<i>Peromyscus leucopus noveboracensis</i>
Mouse, old-field	<i>Peromyscus p. polionotus</i>
Mouse, Oregon pocket	<i>Peromyscus p. parvus</i>
Mouse, parasitic	<i>Peromyscus c. californicus</i>
Mouse, pine	<i>Pitymys sp.</i>
Mouse, pocket	<i>Perognathus sp.</i>
Mouse, red-backed	<i>Evotomys gapperi</i>
Mouse, redwood white-footed	<i>Peromyscus maniculatus rubidus</i>
Mouse, Rocky Mountain jumping	<i>Zapus p. princeps</i>
Mouse, southern California meadow	<i>Microtus californicus sanctidiegi</i>
Mouse, southern parasitic	<i>Peromyscus californicus insignis</i>



Common Name	Scientific Name
Mouse, spiny pocket .....	<i>Perognathus spinatus</i>
Mouse, Tejon pocket .....	<i>Perognathus l. longimembris</i>
Mouse, Townsend meadow .....	<i>Microtus townsendii</i>
Opossum, Florida .....	<i>Didelphis virginiana pigra</i>
Oven-bird .....	<i>Seiurus aurocapillus</i>
Owl, burrowing .....	<i>Speotyto cunicularia hypogaea</i>
Peccary, collared .....	<i>Pecari a. angulatus</i>
Pika, or cony .....	<i>Ochotona</i> sp.
Pika, Rocky Mountain .....	<i>Ochotona p. princeps</i>
Pika, Taylor .....	<i>Ochotona schisticeps taylori</i>
Pocket gopher .....	<i>Thomomys talpoides</i>
Pocket gopher, brown .....	<i>Thomomys f. fuscus</i>
Pocket gopher, Columbia .....	<i>Thomomys columbianus</i>
Pocket gopher, Illinois .....	<i>Geomys illinoensis</i>
Pocket gopher, Nevada .....	<i>Thomomys townsendii nevadensis</i>
Pocket gopher, prairie .....	<i>Thomomys fuscus rufescens</i>
Pocket gopher, Shaw .....	<i>Geomys bursarius</i>
Pocket gopher, southern .....	<i>Geomys floridanus austrinus</i>
Porcupine, yellow-haired .....	<i>Erethizon e. epixanthum</i>
Prairie dog .....	<i>Cynomys</i> spp.
Pyrrhuloxia (gray grosbeak) ....	<i>Pyrrhuloxia sinuata</i>
Quail, bobwhite .....	<i>Colinus virginianus</i>
Quail, California valley .....	<i>Lophortyx californica vallicolla</i>
Rabbit—cottontail, Black Hills ....	<i>Sylvilagus nuttalli grangeri</i>
Rabbit—cottontail, eastern .....	<i>Sylvilagus floridanus mallurus</i>
Rabbit—cottontail, New England ..	<i>Sylvilagus transitionalis</i>
Rabbit—cottontail, Rocky Mountain	<i>Sylvilagus nuttalli pinetis</i>
Rabbit—jack, antelope .....	<i>Lepus californicus alleni</i>
Rabbit—jack, black-tailed .....	<i>Lepus californicus</i>
Rabbit—jack, California .....	<i>Lepus c. californicus</i>
Rabbit—jack, Carolina marsh ....	<i>Sylvilagus p. palustris</i>
Rabbit—jack, Colorado desert ....	<i>Lepus californicus deserticola</i>
Rabbit—jack, Great Plains .....	<i>Lepus californicus melanotis</i>
Rabbit—jack, Florida marsh .....	<i>Sylvilagus palustris paludicola</i>
Rabbit—jack, Idaho pygmy .....	<i>Brachylagus idahoensis</i>
Rabbit—jack, Rocky Mountain	
snowshoe .....	<i>Lepus b. bairdi</i>
Rabbit—jack, snowshoe .....	<i>Lepus</i> sp.
Rabbit—jack, Washington .....	<i>Lepus californicus walla walla</i>
Rabbit—jack, white-tailed .....	<i>Lepus townsendi campanius</i>
Raccoon, Florida .....	<i>Procyon lotor elucus</i>
Raccoon, Hilton Head Island ....	<i>Procyon lotor solutus</i>
Rat, cotton .....	<i>Sigmodon hispidus</i>
Rat, eastern cotton .....	<i>Sigmodon h. hispidus</i>
Rat, domestic .....	<i>Rattus</i> spp.
Rat, Mohave kangaroo .....	<i>Dipodomys mohavensis</i>

Common Name	Scientific Name
Rat, Norway	<i>Rattus norvegicus</i>
Rat, Richardson's kangaroo	<i>Dipodomys ordii richardsoni</i>
Rat, rice	<i>Oryzomys palustris</i>
Rat, roof	<i>Rattus alexandrinus</i>
Rattlesnake, diamond	<i>Crotalus adamanteus</i>
Robin, western	<i>Planesticus migratorius propinquus</i>
Rock squirrel	<i>Otospermophilus</i> sp.
Sheep, mountain	<i>Ovis canadensis</i>
Shrew, Olympic	<i>Sorex setosus</i>
Shrew, short-tailed	<i>Blarina brevicauda</i>
Shrew, Trowbridge	<i>Sorex t. trowbridgii</i>
Shrew, wandering	<i>Sorex v. vagrans</i>
Shrike	<i>Lanius</i> sp.
Skink, red-headed	<i>Eumeces fasciatus</i>
Skunk, Florida	<i>Mephitis elongata</i>
Skunk, long-tailed Texas	<i>Mephitis mesomelas varians</i>
Skunk, Texas hog-nosed	<i>Conepatus leuconotus texensis</i>
Sparrow, Bachman's	<i>Aimophila aestivalis bachmani</i>
Sparrow, chipping	<i>Spizella p. passerina</i>
Sparrow, field	<i>Spizella p. pusilla</i>
Sparrow, Florida grasshopper	<i>Ammodramus savannarum floridanus</i>
Sparrow, fox	<i>Passerella iliaca</i>
Sparrow, golden-crowned	<i>Zonotrichia coronata</i>
Sparrow, Kodiak fox	<i>Passerella iliaca insularis</i>
Sparrow, Nuttall's song	<i>Melospiza m. melodia</i>
Sparrow, rusty song	<i>Melospiza melodia morphna</i>
Sparrow, Savannah	<i>Passerculus sandwichensis</i>
Sparrow, swamp	<i>Melospiza georgiana</i>
Sparrow, Wakulla seaside	<i>Ammodramus maritima juncicola</i>
Sparrow, white-crowned	<i>Zonotrichia l. leucophrys</i>
Sparrow, white-throated	<i>Zonotrichia albicollis</i>
Sparrow, Valdez fox	<i>Passerella iliaca sinuosa</i>
Squirrel, Cascades chickaree	<i>Sciurus douglasii cascadiensis</i>
Squirrel, Douglas chickaree	<i>Sciurus douglasii mollipilosus</i>
Squirrel, fox	<i>Sciurus niger</i>
Squirrel, gray	<i>Sciurus carolinensis</i>
Squirrel, red	<i>Sciurus hudsonicus</i>
Squirrel, Richardson red	<i>Sciurus hudsonicus richardsoni</i>
Squirrel, rock	<i>Otospermophilus grammurus</i>
Squirrel, southern fox	<i>Sciurus n. niger</i>
Squirrel, southern gray	<i>Sciurus c. carolinensis</i>
Squirrel, western	<i>Sciurus griseus</i>
Squirrel, western fox	<i>Sciurus niger rufiventris</i>
Starling	<i>Sternus v. vulgaris</i>
Swallow, violet-green	<i>Tachycineta thalassina lepida</i>
Thrasher, brown	<i>Toxostoma rufum</i>

Common Name	Scientific Name
Thrush, hermit .....	<i>Hylocichla guttata</i>
Thrush, wood .....	<i>Hylocichla mustelina</i>
Tortoise, gopher .....	<i>Gopherus polyphemus</i>
Towhee, red-eyed .....	<i>Pipilo e. erythrophthalmus</i>
Towhee, white-eyed .....	<i>Pipilo erythrophthalmus alleni</i>
Vulture, turkey .....	<i>Cathartes aura septentrionalis</i>
Vulture, black .....	<i>Coragyps a. atratus</i>
Warbler, palm .....	<i>Dendroica palmarum</i>
Waxwing, Bohemian .....	<i>Bombycilla garrula pallidiceps</i>
Waxwing, cedar .....	<i>Bombycilla cedrorum</i>
Weasel .....	<i>Mustela</i> sp.
Weasel, New York .....	<i>Mustela n. noveboracensis</i>
Wolf, red .....	<i>Canis rufus</i>
Wolf, timber .....	<i>Canis gigas</i>
Woodchuck, pallid yellow-bellied ..	<i>Marmota flaviventris avara</i>
Woodchuck, southern .....	<i>Marmota m. monax</i>
Woodchuck, yellow-bellied .....	<i>Marmota f. flaviventris</i>
Woodpecker, red-bellied .....	<i>Centurus carolinus</i>
Wood rat, Baird's .....	<i>Neotoma micropus</i>
Wood rat, bushy-tailed .....	<i>Neotoma cinerea subsp.</i>
Wood rat, Colorado bushy-tailed ..	<i>Neotoma cinerea orolestes</i>
Wood rat, Florida .....	<i>Neotoma f. floridana</i>
Wood rat, large-eared .....	<i>Neotoma fuscipes macrotus</i>
Wood rat, Streater .....	<i>Neotoma fuscipes streatori</i>
Wood rat, western bushy-tailed ...	<i>Neotoma cinerea occidentalis</i>
Wren, Carolina .....	<i>Thryothorus l. ludovicianus</i>
Wren, Florida .....	<i>Thryothorus ludovicianus mamensis</i>
Wren, short-billed marsh .....	<i>Cistothorus stellaris</i>
Yellowthroat, Florida .....	<i>Geothlypis trichas ignota</i>
Yellowthroat, Maryland .....	<i>Geothlypis t. trichas</i>

## REFERENCES

- ARAGÃO, H. DE B. 1911 Notas sobre ixódidas brasileiros. *Memorias d. Inst. Oswaldo Cruz* 3: 145-195.
- BANKS, N. 1908 A revision of the *Ixodoidea*, or ticks, of the United States. *Tech. Ser.* (15) U. S. Dept. Agr. 61 pp.
- BEAUMONT, J. H. 1943 Agricultural science on the war front. *Rept. Hawaii Agric. Exp. Sta.* (1941-42), pp. 47-48.
- BEDFORD, G. A. H. 1913 A new tick to South Africa. 2nd Rep. Dir. Vet. Res. Dept. Agric. Union South Africa: 343-344.
- 1934 South African ticks. I. Onderstepoort J. Vet. Sc. and Animal Ind. 2: 49-99.
- BEQUAERT, J. 1932 *Amblyomma dissimile* Koch, a tick indigenous to the United States (*Acarina: Ixodidae*) *Psyche* 39: 45-47.
- BIRDSEYE, C. 1912 Some common mammals of western Montana in relation to agriculture and spotted fever. *Farmers Bull.* (484) U. S. Dept. Agric. 46 pp.
- BISHOPP, F. C. 1911a Some new North American *Ixodidae* with notes on other species. *Proc. Biol. Soc. Washington* 24: 197-208.
- 1911b The Distribution of the Rocky Mountain Spotted-Fever Tick. *Cir.* (136) U. S. Dept. Agric. 4 pp.
- 1912 A new species of *Dermacentor* and notes on other North American *Ixodidae*. *Proc. Biol. Soc. Washington* 25: 29-38.

- AND HIXSON, HOMER. 1936. Biology and economic importance of the gulf coast tick. *J. Econ. Entom.* 29: 1068-1076.
- AND KING, W. V. 1913. Additional notes on the biology of the Rocky Mountain spotted-fever tick. *J. Econ. Entom.* 6: 200-211.
- AND SMITH, C. N. 1937. A new species of *Ixodes* from Massachusetts. *Proc. Washington Entom. Soc.* 39: 133-138.
- AND SMITH, C. N. 1938. The American dog tick, eastern carrier of Rocky Mountain spotted fever. *Cir.* (478) U. S. Dept. Agric. 25 pp.
- AND WOOD, H. P. 1913. The biology of some North American ticks of the Genus *Dermacentor*. *Parasitology* 6: 153-187.
- BOYNTON, W. H. AND WOODS, GLADYS M. 1933. Deer as carriers of anaplasmosis. *Science* 78: 559-560.
- CHAMBERLIN, W. J. 1937. The ticks of Oregon. *Bull.* (349) Ore. Agric. Exp. Sta. 34 pp.
- COOLEY, R. A. 1908. Preliminary report on the wood tick *Dermacentor* sp. *Bull.* (75) Mont. Agric. Exp. Sta. pp. 95-108.
- 1911. Tick control in relation to the Rocky Mountain spotted fever. *Bull.* (85) Mont. Agric. Exp. Sta. 29 pp.
- 1938. The genera *Dermacentor* and *Otocentor* (*Ixodidae*) in the United States, with studies in variation. *Bull.* (171) Nat. Inst. Hlth. 89 pp.
- 1944. *Ixodes osarkus* n. sp. and *Ornithodoros aquilae* n. sp. with notes on *O. talaje* and *O. kelleyi* (Ixodoidea). *J. Parasitol.* 30: 287-294.
- AND KOHLS, G. M. 1942. *Antricola* new genus, *Amblyomma gertschi* new species, and notes on *Ixodes spinipalpis* (Acarina: Ixodoidea). *Pub. Hlth. Rep.* 57: 1733-1736.
- AND KOHLS, G. M. 1943. *Ixodes californicus* Banks 1904, *Ixodes pacificus* new species and *Ixodes conepti*. *Pan Pac. Entom.* 19: 139-147.
- AND KOHLS, G. M. 1944a. The genus *Amblyomma* (Ixodidae) in the United States. *J. Parasitol.* 30: 77-111.
- AND KOHLS, G. M. 1944b. The Argasidae of North America, Central America, and Cuba. *American Midland Naturalist. Monograph No. 1.* 52 pp. [Notre Dame Univ., Notre Dame, Ind.]
- EDDY, G. W. AND JOYCE, C. R. 1942. Ticks collected on the Tama (Iowa) Indian Reservation with notes on other species. *Iowa State Col. J. Sc.* 16: 539-543.
- FITCH, A. 1872. Fourteenth Rept. on the noxious, beneficial, and other insects of the State of New York. *Trans. N. Y. State Agric. Soc.* (1870) pp. 355-381.
- GIBBONS, R. J. 1939. Survey of Rocky Mountain spotted fever and sylvatic plague in western Canada during 1938. *Canadian Pub. Hlth. J.* 30: 184-187.
- GREEN, R. G., BELL, J. F. AND EVANS, C. A. 1938. Rabbit tick populations on the Lake Alexander area 1931-1938. *Minn. Wildlife Disease Investigations*: 80-86. [Processed.]
- HADWEN, S. 1911. The life history of *Ixodes angustus* Banks. *Proc. Ent. Soc. Brit. Columbia No. 1* (ns) 37-38.
- 1916. In report of the Veterinary Director General for the year ending March 31, 1915. *Sessional Papers No. 15b*: 120-123. Dept. Agric. Canada.
- 1923. Insects affecting livestock. *Bull.* (29 ns) Dept. Agric. Canada. 32 pp.
- HEARLE, E. 1938. The ticks of British Columbia. *Sc. Agric.* 18: 341-354.
- HERMS, W. B. AND HOWELL, D. E. 1935. The western dog tick, *Dermacentor occidentalis* Neum., a vector of bovine anaplasmosis in California. Abstract in *J. Parasitol.* 21: 444.
- HIXSON, H. 1932. The life history and habits of *Ixodes sculptus* Neumann (Ixodidae). *Iowa State Coll. J. Sc.* 7: 35-42.
- HOOKE, W. A. 1908. Life history, habits, and methods of study of the Ixodoidea. *J. Econ. Entom.* 1: 34-51.
- 1909a. Some host relations of ticks. *J. Econ. Entom.* 2: 251-257.
- 1909b. The geographical distribution of American ticks. *J. Econ. Entom.* 2: 403-428.
- , BISHOPP, F. C. AND WOOD, H. P. 1912. The life history and bionomics of some North American ticks. *Bur. Entom. Bull.* (106) U. S. Dept. Agr. 239 pp.
- HUNTER, W. D. AND BISHOPP, F. C. 1911. The Rocky Mountain spotted fever tick with special reference to the problem of its control in the Bitter Root Valley in Montana. *Bur. Entom. Bull.* (105). U. S. Dept. Agr. 47 pp.
- AND HOOKE, W. A. 1907. Information concerning the North American fever tick, with notes on other species. *Bur. Entom. Bull.* (72) U. S. Dept. Agr., 87 pp.
- JOYCE, C. R. AND EDDY, GAINES W. 1943. Host and seasonal notes on the rabbit tick *Haemaphysalis leporis-palustris*. *Iowa State Col. J. Sc.* 17: 205-212.
- KOHL, G. M. 1937. Hosts of the immature stages of the Pacific coast tick *Dermacentor occidentalis* Neum. (Ixodidae). *U. S. Pub. Hlth. Repts.* 52: 490-496.

- MCINTOSH, ALLEN 1931 The brown dog tick. N. Am. Vet. 12: 37-41.
- NEUMANN, L. G. 1899 Révision de la famille des Ixodidés. Mém. Soc. Zool. de France. 12: 107-294.
- 1911 Ixodidae. Das Tierreich (Berlin) 26: 72.
- NEWSTEAD, R. 1909 Reports of the 21st expedition of the Liverpool School of Tropical Medicine, Jamaica 1908-1909. Section I. Medical and economic entomology. Ann. Trop. Med. and Parasitol. 3: 421-469.
- NILES, E. P. 1898 A preliminary study of ticks. (Bull. 86) [n.s. 7(3)] Va. Agric. Exp. Sta., pp. 25-30.
- NUTTALL, G. H. F. AND WARBURTON, C. 1908 Ticks. A monograph of the Ixodoidea. Part I, the Argasidae pp. 1-104 + 34.
- AND ——— 1911 Ticks. A monograph of the Ixodoidea. Part II, the Ixodoidea pp. XIX + 105-348.
- AND ——— 1915 Ticks. A monograph of the Ixodoidea. Part III, the genus *Haemaphysalis* pp. XIII + 349-550.
- PARKER, R. R., BROOKS, C. S. AND MARSH, H. 1929 The occurrence of *Bacterium tularense* in the wood tick *Dermacentor occidentalis*, in California. Pub. Hlth. Reps. 44: 1299-1300.
- , PHILIP, C. B. AND JELLISON, W. L. 1933 Rocky Mountain spotted fever. Am. J. Trop. Med. 13: 341-379.
- PHILIP, C. B. 1933 The occurrence of *Ixodes auritulus* Neum. in North America (Oregon). Science 78: 145-146.
- 1938 A parasitological reconnaissance in Alaska with particular reference to varying hares. II. Parasitological data. J. Parasitol. 24: 483-488.
- REES, C. W. 1934 Transmission of anaplasmosis by various species of ticks. Tech. Bull. (418) U. S. Dept. Agric. 17 pp.
- ROBINSON, L. E. 1926 The genus *Amblyomma*. Ticks. A monograph of the Ixodoidea. Part IV. pp. 1-285.
- SYVERTON, J. T. AND BERRY, G. P. 1936 An arthropod vector for equine encephalomyelitis, western strain. Science 84: 186-187.
- TATE, H. D. 1941 Biology of the tropical cattle tick and other species of tick in Puerto Rico, with notes on the effects on ticks of arsenical dips. J. Agric., Univ. of Puerto Rico 25: 1-24.
- THEILER, A. 1921 Diseases, ticks and their eradication. J. Dept. Agric. Union South Africa, Pretoria, 2: 141-159.
- TWINN, C. R. 1932 Summary of insect conditions in Canada. Ann. Rep. Quebec Soc. for the Protection of Plants. 23 and 24: 149-168.



# NEW MOSQUITO HOSTS FOR *PLASMODIUM GALLINACEUM*<sup>1</sup>

WILLIAM CANTRELL AND HELEN B. JORDAN

From the Department of Bacteriology and Parasitology, the University of Chicago

Mosquito species previously reported to be susceptible to *Plasmodium gallinaceum* include 13 species of *Aedes*, 5 species of *Armigeres* and 2 species of *Culex* (Brumpt 1936, Roubaud, Colas-Belcour and Mathis 1939, Vargas and Beltran 1941, Russell and Mohan 1942, Russell and Menon 1942). These records include only 3 species indigenous to North America, i.e., *Aedes vexans* Meigen, *Aedes aegypti* Linnaeus and *Culex quinquefasciatus* Say.

This report includes the results of observations on 2 American species that have and 7 that have not been previously studied. The mosquitoes were either reared from larvae or captured in the field as adults. They were allowed to feed on an infected chicken and were kept at 28° C. From 3 to 9 days later most of them were dissected and examined for oöcysts. A few were examined for sporozoites and oöcysts after 9 to 21 days.

As may be seen in Table 1, oöcysts were observed in *Aedes campestris* Dyar and

TABLE 1.—Susceptibility of various species of mosquitoes to infection with *Plasmodium gallinaceum*

Species	Source	No. dissected*	No. infected	No. of oöcysts found
Fed on chickens with <i>Plasmodium gallinaceum</i>				
<i>Aedes campestris</i>	Reared from larvae	16	15	130 (mean)
<i>A. stimulans</i>	Wild caught adults	10	6	2, 30, 120, 130
<i>A. triseriatus</i>	Reared from larvae	12	5	1, 25, 32
<i>A. trivittatus</i>	Wild caught adults	7	4	1, 3, 16, 52
<i>A. vexans</i>	Wild caught adults	21	20	607 (mean)
<i>Culex pipiens</i>	Reared from larvae	120	0	
<i>C. salinarius</i>	Reared from larvae	22	3	1, 2, 8
<i>Mansonia perturbans</i>	Wild caught adults	1	1	10
<i>Theobaldia inornata</i>	Reared from larvae	22	1	67
Not fed on chickens infected with <i>Plasmodium gallinaceum</i>				
<i>A. stimulans</i>	Wild caught adults	14	0	
<i>A. trivittatus</i>	Wild caught adults	5	0	
<i>A. vexans</i>	Wild caught adults	10	0	

\*The majority of the mosquitoes were dissected 3 to 9 days after feeding. Four *A. campestris*, 2 *A. stimulans* and 2 *A. triseriatus* were dissected 9 to 21 days after feeding. Since the oöcysts of these mosquitoes had already ruptured, accurate counts could not be made, but sporozoites were found in the salivary glands of all of them.

Knab, *Aedes stimulans* Walker, *Aedes triseriatus* Say, *Aedes trivittatus* Coquillett, *A. vexans*, *Culex salinarius* Coquillett, *Mansonia perturbans* Walker and *Theobaldia inornata* Williston. Sporozoites were observed in the salivary glands of 4 *A. campestris* after 9 to 14 days; in 2 *A. stimulans* after 12 days and in 2 *A. triseriatus* after 21 days. With the exception of *A. vexans*, these species are new hosts for *P. gallinaceum*. The results are in accord with the experiments of other workers in that no species of *Aedes* has been found that is insusceptible to *P. gallinaceum*.

As previously reported by Brumpt, *Culex pipiens* Linnaeus seems to be in-

Received for publication, November 2, 1944.

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

susceptible to infection with *P. gallinaceum* inasmuch as none of 120 mosquitoes became infected. Three of 22 *C. salinarius* became infected.

To rule out the possibility that the wild-caught adults had acquired infections in nature, 10 *A. vexans*, 5 *A. trivittatus* and 14 *A. stimulans*, captured at the same time as were the individuals used for experimental feeding, were dissected without being given an experimental blood meal. No oöcysts were found, as shown in Table 1. Further evidence that the infections were not acquired previous to the experimental blood meal is furnished by the fact that the oöcysts were of approximately the same size and stage of development as oöcysts of the same age in *A. aegypti*.

The values shown in the table for mean numbers of oöcysts should not be taken to indicate comparative susceptibility of the species since the mosquitoes were fed on different chickens. The intensity of the infection in *A. vexans*, however, suggests that this species is particularly susceptible.

Infections were produced in chickens by intramuscular injections of ground salivary glands from *A. campestris* and *A. stimulans*.

#### REFERENCES

- BRUMPT, E. 1936 Étude expérimentale du *Plasmodium gallinaceum* parasite de la poule domestique. Transmission de ce germe par *Stegomyia fasciata* et *Stegomyia albopicta*. Ann. de Parasit. Hum. et Comp. 14: 597-620.
- ROUBAUD, E., COLAS-BELCOUR, J. AND MATHIS, M. 1939 Transmission de *Plasmodium gallinaceum* par *Aedes geniculatus*. Bull. Soc. Path. Exot. 32: 28-30.
- RUSSELL, P. F. AND MENON, P. B. 1942 On the transmission of *Plasmodium gallinaceum* to mosquitoes. Am. J. Trop. Med. 22: 559-563.
- RUSSELL, P. F. AND MOHAN, B. N. 1942 Some mosquito hosts to avian plasmodia with special reference to *Plasmodium gallinaceum*. J. Parasitol. 28: 127-129.
- VARGAS, L. AND BELTRAN, E. 1941 *Culex quinquefasciatus*, a new vector of *Plasmodium gallinaceum*. Science 94: 389-390.

A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS  
*ILLIOSENTIS* (RHADINORHYNCHIDAE)\*

HARLEY J. VAN CLEAVE  
University of Illinois, Urbana

Van Cleave and Lincicome (1939) described *Illiosentis furcatus* as type of a previously unknown genus of the acanthocephalan family RHADINORHYNCHIDAE. The host of this species is *Menticirrhus americanus*, a marine fish of the Gulf of Mexico, belonging to the family SCIAENIDAE, the Croakers. *M. americanus* is the only host species and the Gulf Coast the only region from which *I. furcatus* has been recorded.

In a survey of the worm parasites of the fishes caught by fishermen on the Scripps Institution pier during the spring of 1940, three species of croakers or roncadors (family SCIAENIDAE) were studied intensively. One of these, *Menticirrhus undulatus* (Girard) (locally called the California corbina) was found to be especially heavily infected by a species of the genus *Illiosentis*. Fourteen of 16 individuals carried this worm in numbers ranging from a few to about 200 individuals. The same species of worm was found less frequently and in lighter infections in *Roncador stearnsi* (Steindachner) (the spotfin croaker) and in *Umbrina roncador* Jordan and Gilbert (the yellowfin croaker). Both of these species of fish were caught by line fishermen on the same surf-swept beaches from which the heavily infected *Menticirrhus* were taken. Only 2 of the 4 *Roncador stearnsi* were infected by *Illiosentis* and in these, 4 was the maximum number of worms found in any individual. In 9 specimens of *Umbrina roncador* examined, a single worm was found. In a rather extensive survey of parasites of other fishes in the same region, no other species of fish were encountered as host for *Illiosentis*.

A study of stained permanent mounts demonstrated that the *Illiosentis* from La Jolla is specifically distinct from the only previously known member of the genus. The new species is here described as *Illiosentis cetratus*.

*Illiosentis cetratus* n. sp.  
(Figs. 1-3)

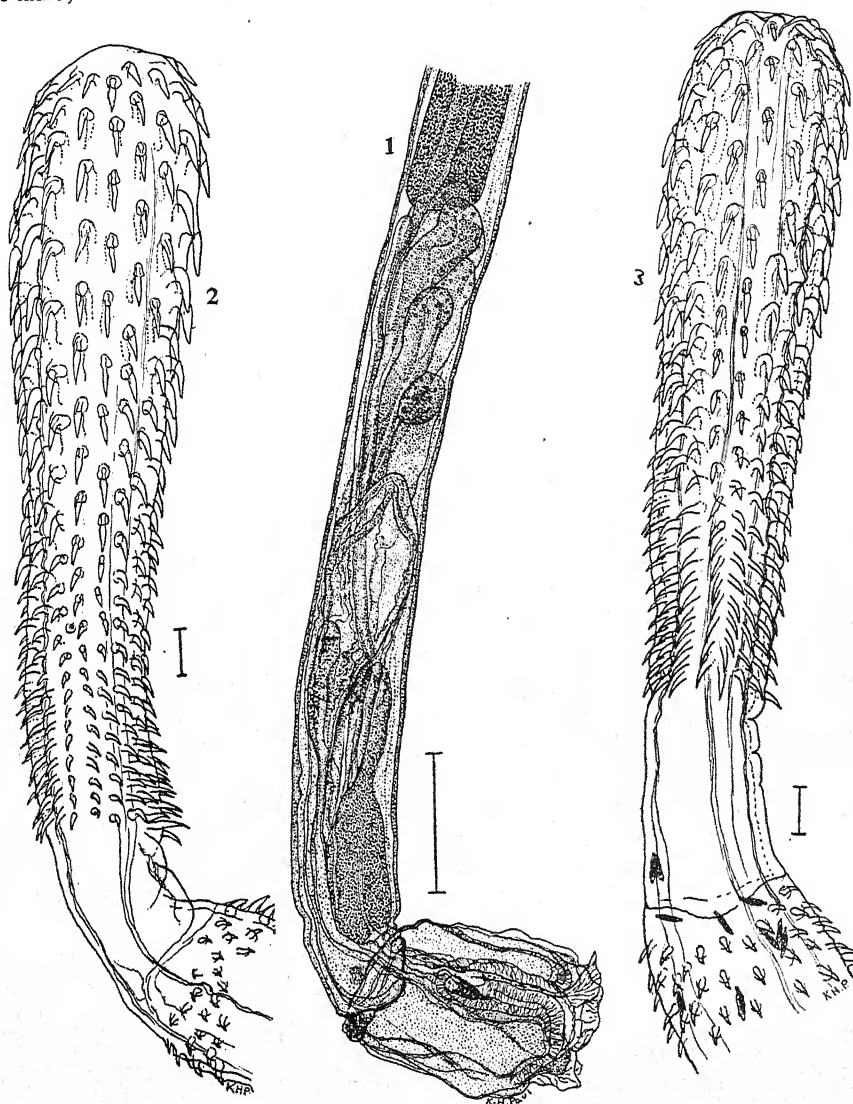
With all of the essential characteristics of the genus *Illiosentis* as enumerated by Van Cleave and Lincicome (1939: 414). Body elongate, flattened in living, turgidly rounded in preserved specimens; preserved females 20 to 37 mm long and 0.55 to 0.77 mm in maximum diameter; males 15 to 27 mm long by 0.44 to 0.66 mm in diameter. The posterior extremity of the body of females has the distinctive cleft in the region of the genital pore as described for *I. furcatus* and internally bears a conspicuous fan-shaped muscular organ bordering the inner margin of the genital cleft as in the genotype. Although on some females there is a pair of lateral body folds adjacent to the pore, a careful examination of several dozen females failed to reveal any papillae or spines such as are distinctive for *I. furcatus*. The observed lateral folds are somewhat similar to those figured for the genital area of *Tegorhynchus* (Van Cleave, 1921). Since the cuticular spines on the genital extremity of the female were cited in the original characterization of the genus *Illiosentis*, their absence in *I. cetratus* stands as evidence that this character has only specific

Received for publication, December 27, 1944.

\*The writer expresses to Director H. U. Sverdrup of the Scripps Institution of Oceanography in La Jolla, California, his appreciation for the many courtesies extended to him during the spring of 1940. Dr. Denis Fox very graciously provided facilities for this study in his laboratory, and Mr. Percy Barnhart was helpful in securing materials for the investigation.

value and should be eliminated from the generic diagnosis. Body spines numerous, restricted to a single uninterrupted zone at the anterior end of the body; commonly 38 to 42  $\mu$  in length.

Proboscis (Figs. 2 and 3) elongate, clavate, unusually somewhat larger in the female than in the male; 1.6 to 2 mm in length by 0.245 to 0.385 mm in diameter. Proboscis hooks quin-



#### EXPLANATION OF PLATE

*Illiosentis cetratus* n. sp.

FIG. 1. Posterior extremity of holotype male (VC 3677.4) showing extruded copulatory bursa. The posterior end of the hind testis is shown at the upper end of the drawing, followed by group of eight intertwined cement glands. Reference line is 0.5 mm long.

FIGS. 2 and 3. Proboscis and a small portion of body proper showing form of proboscis, size and arrangement of hooks and of body spines. A sensory papilla shows in fig. 2 near the base of the eleventh hook from the posterior end of the proboscis. Both figures are of paratype females. Reference lines are 0.1 mm long.

cunxial in arrangement, usually in 16 longitudinal rows with from 19 to 24 (most frequently 20 to 22) hooks in each longitudinal row. A pair of small, rounded, lateral papillae near base

of proboscis, at level of from 8 to 14 hooks from posterior extremity of proboscis. Hooks in basal circle often reaching 70 to 84  $\mu$  on ventral surface, but usually less than 70  $\mu$  on dorsal. On remainder of proboscis hooks are of somewhat variable length, ranging from 42 to 91  $\mu$  near the middle but of more slender form near the anterior tip where they are commonly 49 to 84  $\mu$  long.

Embryos within the body of gravid females, about 58 to 72  $\mu$  long by 8 to 12  $\mu$  in diameter.

In all other anatomical features, including arrangement of the genital organs of the male (Fig. 1), the anatomical features of *I. cetratus* are essentially like those described for *I. furcatus*.

Holotype male (VC 3677.4); allotype female (VC 3680.2) and series of paratypes of both sexes in the collection of H. J. Van Cleave, Urbana, Illinois.

Hosts: mature worms in intestine of *Menticirrhus undulatus* (Girard) and less frequently in *Roncador stearnsi* (Steindachner) and *Umbrina roncadore* Jordan and Gilbert at La Jolla, California. Alternate hosts and developmental stages unknown.

*I. cetratus* differs from *I. furcatus*, the only other species known for the genus *Illiosentis*, most conspicuously in the number and arrangement of the proboscis hooks. The new species has 16 longitudinal rows of 19 to 24 hooks each while the proboscis of *I. furcatus* bears 14 longitudinal rows of 26 to 33 hooks each. Geographical distribution of the two species does not overlap. The new species seems to be restricted to the Pacific coast of southern California while *I. furcatus* has been found only in the Gulf of Mexico.

Acanthocephala are not abundantly represented in the marine fishes of the La Jolla region. During April and May of 1940, the writer conducted a survey of the worm parasites in the marine fishes of that region in which 34 species of fish and a total of more than 190 individuals were examined. Many of the species were represented by only one or a few autopsies, but in the entire survey the only mature acanthocephalans encountered were the specimens of *Illiosentis cetratus* described in this paper.

As was mentioned in the original article describing *I. furcatus*, there have been two earlier references in the literature (Linton, 1905: 398 and Meyer, 1933: 347) to a rhadinorhynchid in *Menticirrhus americanus*. Both of these give the identification, at least tentatively, as *Rhadinorhynchus pristis*. The earlier of these records cites Beaufort, N. C., as the locality. The other reference is less definite, and the identity of the species is questioned by Meyer. The reference in Meyer's monograph is given in the section listing all the species of ACANTHOCEPHALA known to occur in the various species of fish, but in the taxonomic section of the text (pp. 47-56) there is no mention of *Menticirrhus* as host for any species of *Rhadinorhynchus*. The writer has been unable to secure the original material on which these two records are based and so can neither verify nor correct the identifications. The extensive studies of Linton on parasites of fishes of the Atlantic coast have never recorded an acanthocephalan that could be recognized as an *Illiosentis*. Nor has this genus been represented in numerous collections from marine fishes of the Atlantic coast that have come to the writer for study. However, *Rhadinorhynchus tenuicornis* has been often encountered in such collections although it has never been found as yet in *Menticirrhus* of that region.

In spite of the fact that *R. pristis* has been nominally recorded from fishes of the Atlantic coast of North America, there is no recent authoritative verification of the earlier records. Linton (1892) recorded specimens which he deemed to be *Echinorhynchus* (= *Rhadinorhynchus*) *pristis* and in the same publication mentioned a variant to which he applied the name *E. pristis tenuicornis*. In 1918, the present writer showed that the former of these is a distinct and previously unnamed species



to which the name *Rhadinorhynchus ornatus* was applied. In the same revision, the supposed variety of this form was more fully described and was elevated to the status of a distinct species under the name *Rhadinorhynchus tenuicornis*. Following an error in judgment of the author, who referred to *R. tenuicornis* as a new species, several subsequent writers have credited this name to Van Cleave. Since the name was applied as a subspecific name by Linton (1892) in its elevation to specific status the name of the original author should be retained, and the name should be cited *Rhadinorhynchus tenuicornis* (Linton, 1892) Van Cleave, 1918.

An appreciation of the specific distinctness of the two forms which Linton and others erroneously considered as *R. pristis* was strengthened when Chandler (1934) pointed out fundamental morphological differences between them. These differences led him to recognize the genus *Nipporhynchus* which now (Van Cleave and Lincicome, 1940: 179) accommodates *ornatus* while *tenuicornis* remains within the genus *Rhadinorhynchus*. Within recent years numerous collections of Acanthocephala from the Atlantic and from the Gulf of Mexico have been examined critically without discovering *R. pristis* in any of them.

In a preliminary abstract (Van Cleave, 1940) the author emphasized the differences encountered in the parasites of closely related hosts of ACANTHOCEPHALA. The species of the families Rhadinorhynchidae and Gorgorhynchidae offer many interesting problems in the analysis of host-parasite interrelationships. Some species of Acanthocephala with broad geographical range are absent from definite areas within their range, in part at least [as suggested by Chandler (1935: 123) for *R. tenuicornis*] because of the local nature of the distribution of essential intermediate hosts. On the other hand, within a given area where both definitive and intermediate hosts are present, differences in feeding habits of the fish host may be the basis for explanation of differences in incidence of parasitism. This seems the logical explanation for the heavy and general infection by *I. cetratus* in *Menticirrhus undulatus* in an area where *Umbrina roncadore* is virtually free from this parasite.

The occurrence of two distinct but closely related species of *Illiosentis* in two different species of *Menticirrhus*, which are separated by natural barriers, offers the suggestion that speciation within the genus *Illiosentis* has accompanied speciation within the host genus *Menticirrhus*.

#### REFERENCES

- CHANDLER, A. C. 1934 A revision of the Genus *Rhadinorhynchus* (Acanthocephala) with descriptions of new genera and species. *Parasitol.* 26(3): 352-358.  
 ——— 1935 Parasites of fishes in Galveston Bay. *Proc. U. S. Nat. Mus.* 83(2977): 123-157.  
 LINTON, E. 1892 Notes on Entozoa of marine fishes with descriptions of new species. Part III. Acanthocephala. *Rept. Commr. U. S. Comm. Fish and Fisheries for 1888*: 523-542.  
 ——— 1905 Parasites of fishes of Beaufort, N. C. *Bull. U. S. Bur. Fish.* 24: 323-428.  
 MEYER, A. 1932-3 Acanthocephala. *Bronn's Klassen u. Ordnungen des Tierreichs*, 4, Abt. 2, Buch. 2.  
 VAN CLEAVE, H. J. 1918 Acanthocephala of the Subfamily Rhadinorhynchinae. *J. Parasitol.* 5: 17-24.  
 ——— 1921 Acanthocephala collected by the Swedish expedition to the Juan Fernandez Islands (1916-17). *Nat. Hist. Juan Fernandez and Easter Islands* (Uppsala), 3: 75-80.  
 ——— 1940 Some comparisons of Acanthocephala in marine fishes of the Atlantic and Pacific coasts (Abstract). *J. Parasitol.* 26(Suppl. 6): 40-41.  
 ——— AND LINCICOME, D. R. 1939 On a new genus and species of Rhadinorhynchidae (Acanthocephala). *Parasitol.* 31(4): 413-416.  
 ——— AND ——— 1940 A reconsideration of the acanthocephalan family Rhadinorhynchidae. *J. Parasitol.* 26(1): 75-81.

INFLUENCE OF LARVAL TREMATODE INFECTIONS IN SNAILS  
ON THEIR SECOND INTERMEDIATE HOST RELATIONS  
TO THE STRIGEID TREMATODE, *COTYLURUS*  
*FLABELLIFORMIS* (FAUST, 1917)<sup>1</sup>

W. W. CORT, STERLING BRACKETT,<sup>2</sup> LOUIS OLIVIER,<sup>3</sup> AND L. O. NOLF<sup>4</sup>

INTRODUCTION

The present paper will include the data we have accumulated during our studies at the University of Michigan Biological Station on the effect of infections with larval trematodes in lymnaeid snails on their second intermediate host relations to the strigeid trematode, *Cotylurus flabelliformis*. Three of the papers of this series have already dealt with certain aspects of this phase of the subject (Winfield, 1932; Nolf and Cort, 1933; Cort, Olivier and Brackett, 1941). In the four papers of this series already published an account has been given of the life cycle of this trematode, the development of its metacercariae, and the methods used in our studies, which need not be repeated here. For the purpose of comparing the extent of development, the metacercariae counted in our experiments were frequently classified into three arbitrary groups according to the stage of development, viz., (1) "developing" (all stages up to the largest and those beginning to reorganize), (2) "pre-cysts" (those with a distinct hind-body but without a cyst wall, and (3) "cysts" (those that had completed their development and were surrounded by a cyst wall). For the convenience of the reader, the names of the trematode species mentioned in this paper and the pertinent literature on their larval stages are listed in Table 1.

It has been shown in a recently published paper (Cort, Brackett and Olivier, 1944) that the metacercariae of *C. flabelliformis* can complete their development in several different species of lymnaeid snails. When such snails do not harbor the germinal sacs of trematodes, the metacercariae are localized in the hermaphroditic gland which lies imbedded in the digestive gland. In this location they go through their profound metamorphosis from the cercarial body to the encysted metacercarial stage (tetracotyle) in close contact with the host's tissues and feed directly on its substance. When, however, sporocysts or rediae of trematode parasites are present in the digestive gland of the snails, the metacercariae of *C. flabelliformis* develop as hyperparasites inside them. In this location they are cut off completely from the tissues of the snail and must feed on the material absorbed by the germinal sacs and elaborated by them for the nourishment of their developing progeny. Whenever the cercariae of *C. flabelliformis* are able to get into sporocysts or rediae, the metacer-

Received for publication, January 5, 1945.

<sup>1</sup> From the University of Michigan Biological Station and the Department of Parasitology, School of Hygiene and Public Health, The Johns Hopkins University. This paper is the fifth of a series on the second intermediate host relations of *C. flabelliformis* from researches carried on at the University of Michigan Biological Station during the summers of 1932 to 1941. The first paper was by Winfield (1932), the second by Nolf and Cort (1933), the third by Cort, Olivier and Brackett (1941) and the fourth by Cort, Brackett and Olivier (1944).

<sup>2</sup> Now located at the research laboratory of the American Cyanamid Company, Stamford, Connecticut.

<sup>3</sup> Zoölogical Division, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md. At present a Capt. in the Sanitary Corps, A.U.S.

<sup>4</sup> Department of Zoölogy, University of Iowa, Iowa City, Iowa.

cariae almost always develop rapidly to the encysted stage. In fact, as hyperparasites in these germinal sacs they develop more rapidly than in their normal position in the hermaphroditic gland of uninfected hosts.

The presence of larval trematode parasites influences in at least three different ways the ability of the cercariae of *C. flabelliformis* to establish themselves and to develop to the metacercarial stage in snail second intermediate hosts. In the first place, the presence of the sporocysts and developing cercariae of *C. flabelliformis* almost entirely prevents the penetration of the cercariae of this species. In other words, a snail that is serving as an intermediate host of this species cannot be effectively utilized as a second intermediate host. In the second place, the presence of infections with certain other species of larval trematodes partly or, in some cases, almost entirely prevents the use of such infected snails as second intermediate hosts for *C. flabelliformis*. Finally, larval trematode parasites in snails that are not suitable second intermediate hosts for this strigeid may permit its metacercariae to develop, or in partially abnormal hosts they may increase the susceptibility.

TABLE 1.—List of scientific names and pertinent references for the species of trematodes mentioned in this paper

Trematode species	Group	References on larval stages
<i>Cotylurus flabelliformis</i> (Faust, 1917)	Strigeid	Cort and Brooks, 1928; Olivier and Cort, 1941a; Van Haitsma, 1931a
<i>Diplostomum flexicaudum</i> (Cort and Brooks, 1928)	"	Cort and Brooks, 1928; Van Haitsma, 1931b
<i>Cercaria emarginata</i> Cort, 1917	"	Cort, 1917; Olivier and Cort, 1941b
<i>Cercaria yogenae</i> Cort and Brackett, 1937	"	Cort and Brackett, 1937b; Cort, McMullen, and Brackett, 1937
<i>Cercaria luriei</i> Cort and Brooks, 1928	"	Cort and Brooks, 1928; Cort, McMullen, and Brackett, 1937
<i>Cercaria macradena</i> Cort and Brackett, 1938	"	Cort and Brackett, 1938
<i>Schistosomatum douthitti</i> (Cort, 1914)	Schistosome	Price, 1931
<i>Cercaria stagnicola</i> Talbot, 1936	"	Talbot, 1936; Cort, McMullen, and Brackett, 1937
<i>Plagiorchis muris</i> Tanabe, 1922	Plagiorchid	McMullen, 1937
<i>Plagiorchis proximus</i> Barker, 1915	"	McMullen, 1937
<i>Plagiorchis micracanthos</i> Macy, 1931	"	McMullen, 1937
<i>Cercaria talboti</i> McMullen, 1938	"	McMullen, 1938b
<i>Echinostomum revolutum</i> (Froelich, 1802)	Echinostome	Beaver, 1937

#### INFLUENCE OF INFECTIONS WITH THE SPOROCYSTS OF *C. flabelliformis* IN THE VARIETIES OF *Lymnaea stagnalis* AND *Stagnicola emarginata*

It was first shown by Winfield (1932) and later confirmed by Nolf and Cort (1933) that the presence of the daughter sporocysts and developing cercariae of *C. flabelliformis* in snails of the varieties of *L. stagnalis*<sup>5</sup> prevented almost all of the cercariae of this same trematode from penetrating even when such infected snails were exposed to very large numbers. The few cercariae that were able to penetrate into the infected snails, however, entered the daughter sporocysts and developed normally. We have obtained evidence that this same relation also holds for *Stagnicola emarginata angulata* (Sowerby). Adults of this snail variety from a beach near

<sup>5</sup> Two varieties of this species of snail are present in the Douglas Lake region, *L. s. appressa* Say and *L. s. perampla* Walker. They are difficult to distinguish and are intermingled in the area. Both of these varieties were used in the experiments, and no attempt was made to determine to which of them each individual belonged.

Phragmites Flats on Douglas Lake examined during the summer of 1933, harbored unusually large numbers of the metacercariae of *C. flabelliformis*. As shown in Table 2, 27 individuals not infected with larval trematodes contained numbers of metacercariae ranging from 285 to 2921 with an average of 1531. Contrasted with these large numbers, the four snails of this series (Table 2) infected with the sporocysts of *C. flabelliformis* had numbers of metacercariae ranging from 1 to 40 with an average of 21. Earlier in the summer six other individuals of *S. e. angulata* infected with the sporocysts of *C. flabelliformis* were examined from this same beach. They contained numbers of metacercariae varying from 0 to 48 with an average of 15. There is no reason to believe that these 10 snails infected with the sporocysts of *C. flabelliformis* could have in any way escaped the long continued exposure to the cercariae of this trematode which had resulted in such large numbers of metacercariae in other snails on this beach. It is evident, therefore, that in *S. e. angulata*, as well as in the varieties of *L. stagnalis*, the presence of the sporocysts and developing cercariae of *C. flabelliformis* protects the snails from the penetration of the cercariae of this same species.

INFLUENCE OF LARVAL TREMATODE PARASITES, OTHER THAN *C. flabelliformis*,  
IN NORMAL SECOND INTERMEDIATE HOSTS

Nolf and Cort (1933) reported a few experiments on the influence of infections with larval trematodes, other than *C. flabelliformis*, on the development of the metacercariae of *C. flabelliformis* in normal second intermediate hosts. Since this pre-

TABLE 2.—Summary of counts of the metacercariae of *C. flabelliformis* from natural infections in a collection of *S. emarginata angulata* made July 26, 1933, from the "Phragmites Flats Area" on Douglas Lake. The examinations were made from August 1 to 22.  
The infection came from cercariae that had developed in *S. e. angulata*

No. of snails	Larvae trematode infections	Metacercariae of <i>C. flabelliformis</i>		
		Range	Average	Per cent encysted
27	Uninfected	285-2921	1531	49
4	<i>C. flabelliformis</i>	1-40	21	96
7	<i>C. yogenia</i>	755-2072	1292	62
6	<i>C. laruci</i>	419-2425	1592	42
2	<i>C. emarginatae</i>	2296, 2535		85
4	<i>P. proximus</i>	554-1583	1202	80
27	<i>D. flexicaudum</i>	6-2243	963	57
6	<i>P. muris</i>	1-87	23	86
6	<i>E. revolutum</i>	1-150	38	94

liminary work we have been able to add a considerable amount of information on this phase of the problem from the examination of natural infections and from experiments, which will be presented below.

*Data from examinations of natural infections in S. e. angulata.*—Evidence on the influence of the presence of the germinal sacs of other trematodes on the second intermediate host relations of *S. e. angulata* to *C. flabelliformis* came from the series of examinations for natural infections with the metacercariae from the "Phragmites Flats area" already mentioned in this paper and discussed in a previous paper (Cort, Brackett and Olivier, 1944). It was brought out in that paper that the snails on this beach had been exposed for a long period of time to constant infection with the cercariae of *C. flabelliformis* that had developed in the same variety of snail. A summary of the results for the snails examined from this beach is given in Table 2. It can be seen that they were infected with several different species of larval trematodes.



The evidence seemed clear that all of them had had the same type of exposure to the cercariae of *C. flabelliformis* and that any consistent differences in the numbers of metacercariae that they harbored could only be explained on the basis of their larval trematode infections.

It can be seen from Table 2 that the snails of this series of examinations infected with three of the strigeid species (*C. yogena*, *C. laruei*, and *C. emarginatae* and with one of the plagiorchids (*P. proximus*) had numbers of metacercariae comparable to those in the uninfected group. The 27 snails listed in Table 2 that were infected with the sporocysts of *D. flexicaudum* had an average number of metacercariae about two-thirds as great as that of the uninfected group. Also, seven of them had counts below the lowest of that group, the two smallest being 6 and 18 respectively. Finally, the numbers of metacercariae found in the six snails infected with the sporocysts of the other plagiorchid, *P. muris*, and those in the six infected with the rediae of an echinostome, *E. revolutum*, were very small. In fact, the numbers of metacercariae in the snails parasitized by these two species were similar to those in the snails infected with *C. flabelliformis*.

*Experimental studies with S. e. angulata.*<sup>6</sup>—Stimulated by the results of the series of examinations just discussed, we carried out several experiments in which specimens of *S. e. angulata* both unparasitized and parasitized with the germinal sacs of larval trematodes, were exposed to the cercariae of *C. flabelliformis* from *S. e. angulata* and *S. e. canadensis* (Sowerby). A summary of the results of these experiments is given in Table 3. The small numbers of snails in four of these experiments [6 (1933); 15 (1933); 18 (1933); 14 (1935)] that were infected with two of the strigeids, *C. yogena* and *C. laruei*, and with one of the plagiorchids, *P. proximus*, had numbers of metacercariae of *C. flabelliformis* close to the range found in the uninfected groups. The results of the counts for the snails infected with the sporocysts of *D. flexicaudum* were very striking and consistent since in every case the numbers of the metacercariae were very small. In four of the experiments of Table 3 [6 (1933); 15 (1933); 12 (1935); 15 (1935)] there were in all 75 adults of *S. e. angulata* infected with the sporocysts of *D. flexicaudum*, which had been given exposure to the cercariae of *C. flabelliformis* that produced in the uninfected snails of the same experiments average numbers of metacercariae varying from 42 to 374. Of these 75 snails, 57 had no metacercariae, and only 9 of the others had more than 10. It seems evident, therefore, that in experimental infections of this grade, the presence of the sporocysts of *D. flexicaudum* greatly reduced the numbers of the metacercariae that developed. The few that were found were inside the sporocysts and appeared to be developing normally. In the snails infected with *P. muris*, the numbers of metacercariae were also very small and a large proportion of the snails were negative (Table 3). Of 59 snails in three experiments harboring the sporocysts of this trematode, [18 (1933); 14 (1935); 20 (1938)] 35 were negative for metacercariae and only eleven had more than ten. In addition, the counts in Table 3 show that only very small numbers of metacercariae developed in snails infected with the germinal sacs of another plagiorchid, *P. micracanthos* [18 (1933)], an echinostome, *E. revolutum* [14 (1935)], and a schistosome, *C. stagnicolae* [14 (1935)].

<sup>6</sup> To make it easier for the reader to follow the rather complicated relations of the different species of larval trematode parasites to the development of the metacercariae of *C. flabelliformis*, a very condensed summary for this and the later series of experiments is given in Table 7.



*Experiment with Stagnicola palustris elodes* (Say).—Only one of our experiments [4 1937] included specimens of *S. p. elodes* infected with the germinal sacs of larval trematodes (Table 4). The uninfected snails and those parasitized with larval trematodes used in this experiment came from two different collections. As shown in the description of Table 4 both collections had some naturally acquired

TABLE 3.—A summary of the counts of the metacercariae of *C. flabelliformis* in experiments in which uninfected and infected specimens of *S. e. angulata* were exposed to the cercariae of *C. flabelliformis* from *S. e. angulata* [6 (1933); 15 (1933); 18 (1933); 20 (1938)] and from *S. e. canadensis* (Sowerby) [12 (1935); 15 (1935); 14 (1935)]. All the experimental snails were adults except in Experiment 20 (1938).

Control examinations were made as follows for previously acquired natural infections with metacercariae of *C. flabelliformis* of snails from the same collections as these used in the experiments:

12 (1935): 12 *S. e. angulata*, negative  
 15 (1935): 18 *S. e. angulata*, negative  
 14 (1935): 16 *S. e. angulata*, negative  
 20 (1938): 25 *S. e. canadensis*, negative

No control examinations were made for the experiments performed in 1933 [6 (1933); 15 (1933); 18 (1933)]. All the experimental snails used in these three experiments had been naturally infected with small numbers of metacercariae. This was shown by the fact that a few "cysts" were present in them at the time of examination which could not have developed from experimentally introduced cercariae since the time from first exposure to examination was from 9 to 12 days in these experiments. For this reason these cysts are not enumerated in Table 3.

Exper. No.	Days from infection to examination	Larval trematode infections in experimental snails	Data from metacercarial counts			
			No. of snails	No. pos. for metacercariae	Range in numbers of metacercariae	Av. no.
6 (1933)	10	Uninfected	16	16	14-93	42
		<i>C. vogena</i>	2	2	10 & 72	—
		<i>D. flexicaudum</i>	29	2	3 & 3	—
15 (1933)	8-9	Uninfected	5	5	54-705	272
		<i>C. vogena</i>	1	1	130	—
		<i>C. laruei</i>	2	2	17 & 245	—
		<i>D. flexicaudum</i>	20	7	0-81	10
12 (1935)	20-24	Uninfected	6	6	22-443	278
		<i>D. flexicaudum</i>	21	10	0-31	4
15 (1935)	0-12	Uninfected	19	19	81-785	374
		<i>D. flexicaudum</i>	5	3	0-85	29
18 (1933)	8-12	Uninfected	29	28	0-1039	138
		<i>C. laruei</i>	2	2	25 & 68	—
		<i>P. proximus</i>	3	3	2, 29 & 88	—
		<i>P. micracanthos</i>	5	2	0-10	3
		<i>P. muris</i>	22	9	0-90	9
14 (1935)	8-18	Uninfected	15	14	0-464	199
		<i>P. proximus</i>	5	5	56-259	134
		<i>P. muris</i>	30	14	0-40	7
		<i>E. revolutum</i>	8	5	0-11	3
		<i>C. stagnicolae</i>	5	3	0-27	6
20 (1938)	35-36	Uninfected (juv.)	58	35	0-131	25
		<i>P. muris</i>	7	1	2	—

metacercariae of *C. flabelliformis*, which were more numerous in the group from which the parasitized snails were taken. The parasitized group of experimental snails harbored the germinal sacs of three different trematode species, viz., a plagi-orchiid, *C. talboti*, a large echinostome that was not identified, and a strigeid, *C. macradena*. It can be seen from Table 4 that the metacercarial counts for the snails infected with *C. talboti* and the large echinostome were only about one-fourth the size of those of the uninfected group, while those for two snails infected with *C. macradena* were within the range of those for the uninfected group. In interpreting

the counts of metacercariae in this experiment, it must be remembered that all the snails used had had a slight previous exposure to infection. However, the fact that the snails infected with larval trematodes came from the collection that had the larger numbers of naturally acquired metacercariae makes the small numbers of metacercariae found in the experimental snails infected with *C. talboti* and the large echinostome even more significant. It can, therefore, be suggested that the presence of these two larval trematode species in the snails was responsible for the small numbers of metacercariae of *C. flabelliformis* that were able to establish themselves.

TABLE 4.—A summary of the counts of the metacercariae of *C. flabelliformis* in an experiment [4 (1937)] in which uninfected and infected adults of *S. p. clodes* were exposed to cercariae of *C. flabelliformis* from *L. stagnalis*.

Control examinations were made as follows for previously acquired natural infections with metacercariae of *C. flabelliformis* of snails from the same collections as those used in the experiments:

1. Collection from which uninfected experimental snails were taken: 20 snails, six positive with a total of 9 metacercariae.
2. Collection from which infected experimental snails were taken: 19 snails, 13 positive with a total of 119 metacercariae.

Exp. No.	Days from infection to exam.	Larval trematode infections in experimental snails	No. of snails	No. pos. for metacercariae	Data from metacercarial counts				
					Range in number	Av. no.	% "cysts"	% "pre-cysts"	% "developing"
4(1937)	19-22	Uninfected	33	33	6-108	43	66.5	19.5	14.0
	"	<i>C. talboti</i>	25	21	0-48	10	99.2	0.0	0.8
	"	Large echinostome	10	7	0-49	10	81.7	17.3	1.0
	"	<i>C. macradena</i>	2	2	51 & 66	—	100.0	0.0	0.0

#### INFLUENCE OF LARVAL TREMATODE PARASITES ON THE SUSCEPTIBILITY OF ABNORMAL SECOND INTERMEDIATE HOSTS

A large part of our experimental studies on this particular phase of the problem has already been published (Cort, Olivier and Brackett, 1941). It was reported in that paper that physid and planorbid snails are abnormal second intermediate hosts for the cercariae of *C. flabelliformis*. The cercariae penetrated into the snails belonging to these two families but almost no development took place unless they were infected with certain species of larval trematodes. In such parasitized snails the cercariae penetrated into the germinal sacs of the trematodes and developed, frequently in large numbers, as hyperparasites. In fact, the metacercariae completed their development under these conditions in a shorter time than in lymnaeid snails, not infected with larval trematodes, that were used in the experiments as normal second intermediate host controls.

In an earlier paper (Cort, Brackett and Olivier, 1944) it has been shown that *L. stagnalis* is almost a completely abnormal second intermediate host for the variety of *C. flabelliformis*, the cercariae of which develop in *S. emarginata*. Also, *S. emarginata*, especially in the juvenile stage, is abnormal in its second intermediate host rela-

tions to the variety of *C. flabelliformis* the cercariae of which develop in *L. stagnalis*. It seemed of interest, therefore, to determine whether the presence of the germinal sacs of larval trematodes would make these snails more susceptible to the metacercarial stages of the varieties of *C. flabelliformis* that do not develop normally in them.

*Experiments with L. stagnalis exposed to cercariae of C. flabelliformis from S. emarginata.*—The information on this relation in *L. stagnalis* is rather meager. In one of the experiments [5 (1936)] (not included in the tables of this paper) in which this host was exposed to cercariae of *C. flabelliformis* from *S. emarginata*, some of the experimental snails were infected with the sporocysts of *S. douthitti* and others with those of *D. flexicaudum*. The snails in this experiment were exposed to the cercariae from two infected specimens of *S. e. angulata* for a period of ten days. On examination over five weeks after the first exposure to the cercariae the nine snails without larval trematode parasites had numbers of metacercariae ranging from 0 to 300 with an average of 40, of which 77.9 per cent were "cysts," 6.6 per cent "pre-cysts," and 15.5 per cent developing. However, five of these nine snails were entirely negative for metacercariae, two had very small numbers (1 and 7) and only two had larger numbers (54 and 300). Only two of the five snails infected with the sporocysts of *S. douthitti* had metacercariae and then in only very small numbers (1 and 11). However, all the seven snails in this experiment infected with the sporocysts of *D. flexicaudum* harbored metacercariae varying in numbers from 6 to 141 with an average of 44, of which 45.9 per cent were "cysts," 28.7 per cent "pre-cysts," and 25.4 per cent "developing." It is not possible to be sure that there were not some naturally acquired metacercariae in the snails in this experiment because only two individuals of the same collections were examined for metacercariae. These two were negative. At any rate, it is perfectly evident that the presence of the sporocysts of *S. douthitti* in no way increased the susceptibility of the snails. On the other hand, the presence of the sporocysts of *D. flexicaudum* seemed to increase the susceptibility of the snails that harbored this trematode.

*Experiments with juveniles of S. emarginata exposed to the cercariae of C. flabelliformis from L. stagnalis.*—The experiments in which juveniles of *S. emarginata* were exposed to cercariae of *C. flabelliformis* from *L. stagnalis* [21 (1938); 1A and 1B (1941); 2 (1941)] gave clear-cut results (Table 5). In these experiments as shown in the table and discussed in the earlier paper (Cort, Brackett, and Olivier, 1944) there was almost no normal development of the metacercariae in the snails not parasitized with larval trematodes. These results were interpreted as indicating that the juveniles of *S. emarginata* are almost completely abnormal hosts for the variety of *C. flabelliformis*, the cercariae of which develop in *L. stagnalis*. The presence of the sporocysts of trematodes changed this relation completely. In the first experiment of Table 5 the six juveniles of *S. e. canadensis* infected with the sporocysts of *D. flexicaudum* had an average of 124 metacercariae almost all of which were encysted, while the 73 unparasitized snails had an average of only 23 metacercariae, hardly any of which had even started to develop. In experiment 1A (1941) the results are even more striking since none of the 26 uninfected juveniles of *S. e. angulata* had any metacercariae at all while the numbers in the five snails infected with the sporocysts of *P. muris* were almost as large as in the much larger uninfected adults of *S. p. elodes* that had had the same exposure to the cercariae. In experiment 1B (1941) the results are equally clear cut since only one of the 25 unin-

TABLE 5.—Metacercarial counts in experiments in which juveniles of *S. emarginata* were exposed to the cercariae of *C. flabelliformis* from *L. stagnalis*. The following numbers of snails were examined from the same collections as those used in the experiments for previously acquired natural infections and all were negative for the metacercariae of *C. flabelliformis*: 21 (1938), 25; 1A and B (1941), 20; 2 (1941) at least 100.

Exp. No.	Days from infection to examination	Larval trematode infections in experimental snails	Data from metacercarial counts						
			No. of snails	No. pos. for metacercariae	Range in no.	Av. no.	% "cysts"	% "pre-cysts"	% "developing"
21 (1938)	36-37	<i>S. e. canadensis</i> (juv.)—uninfected	73	47	0-184	23	99.0	...	99.0
1A (1941)	"	<i>S. e. canadensis</i> (juv.)— <i>D. flexicaudum</i>	6	5	0-271	124	...	...	...
	22	<i>S. p. elodes</i> (adult)—uninfected	4	4	14-127	49	64.9	35.1	0.0
	"	<i>S. e. angulata</i> (juv.)—uninfected	26	0	...	0	...	...	...
	"	<i>S. e. angulata</i> (juv.)— <i>P. muris</i>	5	5	21-80	43	69.4	14.8	15.8
1B (1941)	29	<i>S. p. elodes</i> (adult)—uninfected	5	5	4-54	38	95.8	4.2	0.0
	"	<i>S. e. angulata</i> (juv.)—uninfected	25	1	6	...	...	...	...
	"	<i>S. e. angulata</i> (juv.)— <i>P. muris</i>	10	8	0-263	47	98.1	1.5	0.4
	"	<i>S. e. angulata</i> (juv.)— <i>D. flexicaudum</i>	5	5	4-36	20	100.0	0.0	0.0
2 (1941)	16-18	<i>B. megasoma</i> (adult)—uninfected	7	7	3-51	16	0.0	0.0	100.0
	"	<i>S. e. angulata</i> (juv.)—uninfected	23	18	0-48	82	0.0	0.0	100.0
	"	<i>S. e. angulata</i> (juv.)— <i>P. muris</i>	9	9	7-214	18	0.0	96.4	3.6
	"	<i>S. e. angulata</i> (juv.)— <i>P. proximus</i>	1	1	184	...	0.0	91.2	9.8
		<i>S. e. angulata</i> (juv.)— <i>D. flexicaudum</i>	2	2	48 & 398	...	0.0	76.0	24.0

infected juveniles had any metacercariae, and then a very small number, while the average for the 10 juveniles of *S. e. angulata* infected with the sporocysts of *P. muris* was considerably larger than for the adults of *S. p. elodes*. The 5 snails infected with the sporocysts of *D. flexicaudum* in this experiment had somewhat smaller numbers of metacercariae, all of which were encysted. Finally in experiment 2 (1941) the uninfected juveniles of *S. e. angulata* had an average of 18 metacercariae almost all of which had hardly started development at all, while the snails infected with the sporocysts of *P. muris*, *P. proximus* and *P. flexicaudum* had much larger numbers which were developing normally. Taken together the results of these four experiments clearly show that the presence of the germinal sacs of these trematodes in juveniles of *S. emarginata*, which when uninfected were almost completely abnormal second intermediate hosts for the cercariae of *C. flabelliformis* from *L. stagnalis*, permitted the normal development of considerable numbers of the metacercariae.

*Experiments with adults of S. emarginata exposed to the cercariae of C. flabelliformis from L. stagnalis.*—As suggested in the earlier paper (Cort, Brackett, and Olivier, 1944) and shown in Table 6, adults of *S. e. angulata*, when exposed to the cercariae of *C. flabelliformis* from *L. stagnalis* show less abnormality in their second intermediate host relations than juveniles of the same species, since frequently a considerable number of metacercariae developed normally in them. It was brought out, however, that the numbers of metacercariae that developed were considerably less than in adults of *S. emarginata* that were exposed to cercariae of *C. flabelliformis* from this same host (cf. Tables 3 and 6).

It was of interest, therefore, to see whether adults of *S. emarginata* that were infected with the germinal sacs of larval trematodes would take larger numbers of the metacercariae than the unparasitized snails of the same species when exposed to the cercariae of *C. flabelliformis* from *L. stagnalis*. Table 6 gives the metacercarial counts from the experiments of our series that give information on this point. It can be seen from this table that the adults of *S. e. angulata* in the experiments that harbored the sporocysts of three of the strigeid species (*C. emarginatae*, *C. yogena*, and *C. laruei* [13 (1935); 5B (1937); 5D (1937); 5E (1937)]) had very much larger numbers of metacercariae than the unparasitized specimens of *S. e. angulata* in the same experiments. The number of snails infected with these three strigeids is rather small in any particular experiment but the differences are striking and consistent. In the four experiments taken together, the 17 snails infected with these three strigeids had numbers of metacercariae ranging from 11 to 467 with an average of 112; while the 33 unparasitized specimens of *S. e. angulata* in the same experiments had numbers ranging from 1 to 71 with an average of 21. In two of the experiments [5B (1937); 5D (1937)] two adults of *S. e. emarginata* infected with *C. laruei* and *C. emarginatae* respectively had larger numbers of metacercariae (261 and 467) than any of the much larger specimens of *L. stagnalis* in the same experiments.

It also can be seen from Table 6 [5B (1937); 5D (1937); 5E (1937)] that adults of *S. e. angulata* infected with the schistosome, *C. stagnicola*, after exposure to the cercariae of *C. flabelliformis* from *L. stagnalis*, had much larger numbers of metacercariae than the uninfected snails of the same species in the same experiments. While the number of snails in the experiments infected with this schistosome is small, the results are consistent and the differences are considerable. The nine snails infected with *C. stagnicola* had numbers of metacercariae ranging from 17 to 82 with



TABLE 6.—A summary of the counts of metacercariae in experiments in which uninfected and infected adults of *S. e. angulata* were exposed to the cercariae of *C. flabelliformis* that had developed in *L. stagnalis*. A few specimens of *L. stagnalis* were placed in each of these experiments as controls of the extent of exposure to the cercariae.

Control examinations were made as follows for previously acquired natural infections with the metacercariae of *C. flabelliformis* of snails from the same collections as those used in these experiments:

13 (1935) : 15 *L. stagnalis*, negative; *S. e. angulata*, no examination, but no suggestion of previous infection from experimental results.

5B, C, D, E, (1937) : 50 *L. stagnalis*, 47 negative, 3 positive with a total of 5 "cysts"; 23 *S. e. angulata*, 22 negative, 1 positive with 1 "cyst."

Exper. No.	Days from infection to examination	Larval trematode infection in experimental snails	Data from metacercarial counts							
			No. of snails	No. pos. for metacercariae	Range in no.	Av. no.	% "cysts"	% "pre-cysts"	% "developing"	
13 (1935)	14-17	<i>L. stagnalis</i> —uninfected	3	3	169-394	283	10.6		89.4	87.9
	"	<i>S. e. angulata</i> —uninfected	11	11	1-71	24	6.4	5.7		0.0
	"	<i>S. e. angulata</i> — <i>C. emarginatae</i>	3	3	45-102	79	0.8	98.2		0.0
	"	<i>S. e. angulata</i> — <i>C. yongei</i>	2	2	53 & 56	...	63.3	36.7		0.0
5B (1937)	18-19	<i>L. stagnalis</i> —uninfected	6	6	18-240	103	14.5	27.9		67.6
		<i>S. e. angulata</i> —uninfected	10	10	3-51	22	48.4	30.9		20.7
	"	<i>S. e. angulata</i> — <i>C. emarginatae</i>	2	2	109 & 111	...	29.5	70.5		0.0
	"	<i>S. e. angulata</i> — <i>C. laruet</i>	2	2	97 & 261	...	64.4	32.3		0.3
	"	<i>S. e. angulata</i> — <i>C. yongei</i>	2	2	11 & 107	...	61.1	34.7		4.2
	"	<i>S. e. angulata</i> — <i>C. stagnicola</i>	2	2	58 & 65	...	25.2	71.5		3.3
	"	<i>S. e. angulata</i> — <i>D. flexicaudum</i>	4	4	0-6	4	50.0	50.0		0.0
	"	<i>L. stagnalis</i> —uninfected	3	3	59-158	98	74.6	22.0		3.4
5C (1937)	"	<i>S. e. angulata</i> —uninfected	5	5	3-55	21	21.9	41.9		36.2
	"	<i>S. e. angulata</i> — <i>D. flexicaudum</i>	5	5	1-4	1	70.0	30.0		0.0
	"	<i>S. e. angulata</i> — <i>P. muris</i>	6	4	0-29	7	97.5	0.0		2.5
	"	<i>L. stagnalis</i> —uninfected	6	6	75-172	104	59.2	30.1		10.7
5D (1937)	"	<i>S. e. angulata</i> —uninfected	8	8	1-47	23	38.7	25.7		35.6
	"	<i>S. e. angulata</i> — <i>C. emarginatae</i>	3	3	31-467	207	12.4	0.8		6.8
	"	<i>S. e. angulata</i> — <i>C. stagnicola</i>	3	3	17-82	68	72.8	20.0		5.2
	"	<i>S. e. angulata</i> — <i>P. muris</i>	8	4	0-19	4	91.4	8.6		0.0
5E (1937)	21-22	<i>L. stagnalis</i> —uninfected	4	4	103-221	147	46.6	28.6		24.8
	"	<i>S. e. angulata</i> —uninfected	4	4	1-6	4	57.2	35.7		7.1
	"	<i>S. e. angulata</i> — <i>C. emarginatae</i>	3	3	24-183	78	80.4	17.0		2.6
	"	<i>S. e. angulata</i> — <i>C. stagnicola</i>	4	4	25-52	34	68.4	30.9		0.7

an average of 51, while the 22 uninfected specimens of *S. e. angulata* in the same experiments had numbers ranging from 1 to 51 with an average of 19.

In three of the experiments summarized in Table 6 there were included adults of *S. e. angulata* infected with the germinal sacs of *D. flexicaudum* and *P. muris* [5B (1937); 5C (1937); 5D (1937)]. The snails parasitized by these two trematode species had very few metacercariae. In fact, the numbers were so small that it appeared as if the presence of these two trematodes had definitely decreased the susceptibility of the snails to the infection with the cercariae of *C. flabelliformis* from *L. stagnalis*. However, the evidence given above clearly shows that parasitism with the other four larval trematodes (*C. emarginatae*, *C. yogenae*, *C. laruei* and *C. stagnicolae*) had definitely increased the susceptibility of the snails to this variety of *C. flabelliformis*.

#### DISCUSSION

One of the most interesting of the second intermediate host relationships of *C. flabelliformis* is the development of its metacercariae inside the germinal sacs of the trematode parasites of the snails serving as second intermediate hosts. In this location they are able to develop as hyperparasites cut off completely from the host's tissues. That they find the food substances absorbed by the germinal sacs very suitable for their nutrition is shown by their rapid development to the encysted stage. The presence of strigeid metacercariae inside sporocysts and rediae of other trematode parasites was described and figured by some of the earliest observers on larval trematodes. Such a relation is characteristic of the few strigeid species that normally utilize snails as second intermediate hosts. It is a true hyperparasitism of one species of parasite inside of another. It, therefore, is quite a different relation from those much more numerous cases in which trematode cercariae precociously develop into metacercariae without leaving their own germinal sacs (Cort and Brackett, 1937a; McMullen, 1938a). As noted above, the metacercariae of *C. flabelliformis* practically always develop more rapidly as hyperparasites inside the germinal sacs of trematodes than in their normal location in unparasitized snails. This has been repeatedly observed, both in normal and abnormal hosts, whenever the experiments permitted comparisons.

As brought out in the experiments, the presence of the germinal sacs of trematodes in snails influences in several different ways their second intermediate host relations to *C. flabelliformis*. In the first place, when snails are infected with the sporocysts and developing cercariae of *C. flabelliformis*, they are almost completely protected against the development of its metacercariae. This relation seems to be a specific immunity produced by one stage of a parasite against a later stage. In the second place, normal second intermediate hosts of *C. flabelliformis* are protected against the development of its metacercariae by the presence of the germinal sacs and developing cercariae of certain other trematode species. This relation might be considered as a nonspecific immunity produced in the snails by the presence of these parasites. In the third place, the presence of certain species of larval trematodes permits the development of the metacercariae of *C. flabelliformis* in snails that are entirely or partially unsuitable second intermediate hosts for this species of trematode. Finally, the presence of certain particular species of larval trematode parasites produced both a nonspecific immunity in normal second intermediate hosts and also increased the susceptibility in abnormal hosts. Further discussion of these relations follows.

*Specific immunity.*—Perhaps most striking of all is the protection which the sporocysts and developing cercariae of *C. flabelliformis* gives the infected snails against its own metacercarial stage. As already stated, this appears to be a specific immunity produced in the snail host by one stage of the parasite's life cycle that protects it against a later stage. This immunity is not absolute since a very few metacercariae do develop normally inside the sporocysts. What happens is that when a snail infected with the sporocysts of *C. flabelliformis* is exposed to the cercariae of this same species, most of them do not penetrate at all. Their reactions in such exposures have been observed under a low-power binocular microscope. When the cercariae of *C. flabelliformis* came into contact with the body surfaces of specimens of *L. stagnalis* infected with the sporocysts of the same species, they reacted entirely differently from those that came into contact with uninfected controls (Winfield, 1932; Cort and Nolf, 1933). In the case of the infected snails, the cercariae moved along the surface with a looping movement and soon swam away. With the uninfected snails they started to penetrate almost immediately on making contact, and soon their bodies disappeared into the snail's tissue leaving behind only their detached tails. This shows that the specific immunity produced by the presence of the sporocysts and developing cercariae of *C. flabelliformis* in some way prevents most of the cercariae of this species from penetrating into the infected snails. Penetration of the cercariae of *C. flabelliformis* into snails is not a host specific phenomenon since they penetrate readily into abnormal second intermediate hosts. Also, there is not the slightest evidence that suitable second intermediate hosts exert any attraction for the cercariae as is the case with the intermediate hosts for the miracidia. Therefore, the specific immunity which prevents the penetration of the cercariae must be caused by some change, probably chemical, in the tissues of the snail, produced by the presence of the sporocysts and developing cercariae of *C. flabelliformis*, which actually repels the cercariae of this species when they make contact.

*Nonspecific immunity.*—In snails that are normal second intermediate hosts for *C. flabelliformis*, the presence of the germinal sacs of certain trematode species is associated with a great reduction in the numbers of metacercariae that develop. Although our evidence is not conclusive, this reduction in numbers of metacercariae in most cases seems to be due to the presence in the snails infected with these larval trematode species of some sort of a nonspecific immunity that prevents the penetration of the cercariae. Like the specific immunity produced by the presence of the sporocysts of *C. flabelliformis*, this nonspecific immunity is not absolute since a small proportion of the cercariae were able to penetrate and develop normally into encysted metacercariae inside the germinal sacs.

Of the trematode species that produce this nonspecific immunity the relations of *D. flexicaudum* are particularly interesting. The presence of the sporocysts of this trematode in specimens of *S. emarginata* in experiments produced a definite nonspecific immunity to the cercariae of *C. flabelliformis* from this host (Table 3). When, however, snails of this species infected with *D. flexicaudum* had long continued heavy exposure to the cercariae of this variety under natural conditions, large numbers of metacercariae developed in most of them, indicating that the nonspecific immunity was broken down (Table 2).

Of the other species of larval trematodes that produce nonspecific immunity in normal second intermediate hosts, we were able to obtain the most extensive infor-

mation on *P. muris*. Specimens of *S. emarginata* infected with the sporocysts of this trematode had only a few metacercariae when exposed to long continued natural infection (Table 2) with the cercariae of *C. flabelliformis* from the same snail species. Also, 65 snails of the same species, infected with the larval stages of this species of trematode showed without exception the same type of resistance when exposed in three different experiments (Table 3) to the cercariae of *C. flabelliformis* from *S. emarginata*. The evidence of nonspecific immunity in individuals of *S. emarginata* infected with the rediae of *E. revolutum* also rests both on their resistance to natural and experimental exposure to the cercariae. The evidence for a nonspecific immunity produced by infections with *P. micranthos* and *C. stagnicolae* in *S. emarginata* and by *C. talboti* and the unidentified echinostome in *S. p. elodes* in each case comes from a single experiment. In each of these cases, however, the differences in numbers of metacercariae between the snails infected with these trematodes and the uninfected groups were very striking.

In all these instances of nonspecific immunity to the metacercarial stages of *C. flabelliformis* produced by infections with the species of trematodes listed above, the numbers of metacercariae were greatly reduced but the few that got into the germinal sacs developed normally. Our view in regard to the mechanism of this non-specific immunity is that it is probably similar to that of the specific immunity produced by infections with the sporocysts of *C. flabelliformis*. There appears to be a great reduction in the numbers of cercariae of *C. flabelliformis* that penetrate into the snails infected with these particular species of larval trematodes, produced perhaps by some chemical change that repels the cercariae.

It is evident, also, that the nonspecific immunity produced against the penetration of the cercariae of *C. flabelliformis* by the presence of certain species of larval trematodes in normal second intermediate hosts is a species and not a group reaction. It was produced by the presence of three plagiorchiid species, *P. muris*, *P. micranthos* and *C. talboti*, but not by another closely related species, *P. proximus*; and it was produced by only one, *D. flexicaudum*, of several species of strigeids that were present in the snails in the experiments.

*Increased susceptibility of abnormal hosts produced by larval trematode infections.*—When snails are abnormal second intermediate hosts for *C. flabelliformis* the presence of larval trematode infections produces an entirely different effect from that discussed above in normal hosts. A discussion of this relation in physid and planorbid snails has already been published (Cort, Olivier, and Brackett, 1941). When physid and planorbid snails were not parasitized by larval trematodes and were exposed to the cercariae of *C. flabelliformis*, they penetrated in large numbers, but except in a very few, and perhaps questionable instances, did not even start to develop. When, however, larval trematode infections were present, large numbers of the cercariae were able to penetrate into their germinal sacs and develop normally to the encysted metacercarial stage. It was suggested that when the bodies of the cercariae that penetrate into abnormal hosts are able to get into the sporocysts or rediae of the trematode parasites, they are protected from any immune reactions of the abnormal hosts tissues, and find very satisfactory for their nutrition the food material which has been absorbed by the germinal sacs and prepared for the nourishment of their own progeny. Thus the presence of the germinal sacs of trematode parasites permits *C. flabelliformis* to use these abnormal snail hosts for the development of its metacercariae and greatly extends its range of second intermediate hosts.



The discovery that *L. stagnalis* is a distinctly abnormal host for the variety of *C. flabelliformis*, the cercariae of which develop in *S. emarginata*, gave a chance to see whether in this host the presence of larval trematodes would permit the development of the metacercariae of this variety. In one experiment it was found that almost no development of metacercariae occurred in snails of this species infected with the sporocysts of *S. douthitti*, while in those infected with the sporocysts of *D. flexicaudum* a considerable number of the metacercariae developed normally inside the germinal sacs. We can give no explanation for the difference in the results with these two parasites. The presence of the sporocysts of *S. douthitti* was found in earlier experiments (Nolf and Cort, 1933; Tables 4 and 5) to interfere with the normal development in *L. stagnalis* of the cercariae of *C. flabelliformis* of the variety from *L. stagnalis*.

As noted above (Table 5) juveniles of *S. emarginata* are distinctly abnormal second intermediate hosts for the variety of *C. flabelliformis* the cercariae of which develop in *L. stagnalis*. There was evidence from the experiments that the cercariae of this variety penetrated into the juveniles, just as they did into physid and planorbid snails, but were unable to develop normally. When, however, the juveniles of this species were infected with larval trematode parasites (Table 4) large numbers of metacercariae developed normally inside the germinal sacs. It can be seen from Table 5 that except for one snail infected with the sporocysts of *P. proximus*, the larval trematode parasites of this series were either *D. flexicaudum* or *P. muris*. The presence of both these trematode parasites appeared to break down completely the abnormal host resistance of the juveniles of *S. emarginata* since large numbers of metacercariae developed inside their germinal sacs.

As brought out previously, adults of *S. emarginata* appear to have lost a considerable part of the abnormal host resistance which the juveniles of this species exhibit so definitely to the variety of *C. flabelliformis*, the cercariae of which develop in *L. stagnalis*. However, they appear to be somewhat abnormal second intermediate hosts to this variety since when the cercariae of *C. flabelliformis* used to infect them came from *S. emarginata* larger and more consistent infections of metacercariae were produced than when they came from *L. stagnalis* (cf. Tables 3 and 6). Of special interest, therefore, are the experiments showing the effect of larval trematode infections in adults of *S. emarginata* on the numbers of metacercariae that developed when they were exposed to the cercariae of the variety of *C. flabelliformis* from *L. stagnalis*. These experiments show (Table 6) that infections with the sporocysts of four different species of larval trematodes (*C. emarginatae*, *C. yogenae*, *C. laruei*, and *C. stagnicolae*) definitely increased the susceptibility of adults of this snail species to this variety of *C. flabelliformis*, since the numbers of metacercariae that developed in the infected snails were much greater than in the uninfected snails that had the same exposure to the cercariae. On the other hand, in experiments where the cercariae of *C. flabelliformis* were of the variety that develops in *S. emarginata*, the adults of *S. emarginata* infected with these same species of larval trematodes never had larger numbers of metacercariae than the uninfected snails (Table 3). It is of special interest to point out that adults of *S. emarginata* infected with the sporocysts of *C. flexicaudum* and *P. muris* showed the same nonspecific immunity to the development of the metacercariae of *C. flabelliformis* when the cercariae came from *L. stagnalis* as when they came from *S. emarginata* (cf. Tables 3 and 6).



TABLE 7.—Summary of the results on the development of the metacercariae of the two varieties of *C. flabelliformis* in lymnaeid snails infected with larval trematodes

	Uninfected	<i>C. flabelliformis</i>	<i>C. emarginata</i>	<i>C. yoyena</i>	<i>C. larnei</i>	<i>C. macradena</i>	<i>D. flexicaudum</i>	<i>C. stagnicolae</i>	<i>S. douvillei</i>	<i>P. proximus</i>	<i>P. muris</i>	<i>P. microcanthos</i>	<i>C. talboti</i>	<i>E. revolutum</i>	Large echinostome
A. Variety of <i>C. flabelliformis</i> , the cercariae of which develop in <i>S. emarginata</i>															
<i>L. stagnalis</i> .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. emarginata</i> (juveniles) .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. emarginata</i> (adults) .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B. Variety of <i>C. flabelliformis</i> , the cercariae of which develop in <i>L. stagnalis</i>															
<i>L. stagnalis</i> .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. palustris clodes</i> .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. emarginata</i> (juveniles) .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. emarginata</i> (adults) .....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = good development; ± = moderate development; - = very little development.

*Relations of individual species of larval trematodes.*—Table 7 is a very condensed summary of the effects of parasitism with individual species of larval trematodes in the lymnaeid snails included in our experimental studies on the development of the metacercariae of the two varieties of *C. flabelliformis*. It can be seen from this table that the effects of three of the species (*C. flexicaudum*, *C. stagnicolae* and *P. muris*) are rather complicated and appear to be inconsistent. All three species appear to produce a nonspecific immunity to the development of the metacercariae of *C. flabelliformis* when they are present in snails that are normal second intermediate hosts. In distinctly abnormal hosts (*L. stagnalis* to the variety of *C. flabelliformis* from *S. emarginata* and juveniles of *S. emarginata* to the variety from *L. stagnalis*) the presence of two of them (*C. flexicaudum* and *P. muris*) completely broke down the resistance and allowed large numbers of metacercariae to develop. Finally, in adults of *S. emarginata* which are only slightly abnormal hosts to the variety of *C. flabelliformis* from *L. stagnalis* the presence of two of them (*C. flexicaudum* and *P. muris*) produced an almost complete nonspecific immunity while the presence of the other (*C. stagnicolae*) increased the susceptibility. However, further studies are needed to check the relationship of infections with *C. stagnicolae* since the evidence for its production of nonspecific immunity in a normal host rests on only one experiment including 5 individuals [Table 3, 14 (1935)]. The evidence on the relations of *C. flexicaudum* and *P. muris*, on the other hand, rests on a considerable series of different experiments involving large numbers of individuals. Therefore, it seems possible to conclude that the presence of infections with these two species produces both a specific immunity in normal and slightly abnormal hosts, and also breaks down the abnormal host resistance in distinctly abnormal hosts.

#### SUMMARY

This paper brings together all the data obtained during the summers of 1932 to 1941 at the University of Michigan Biological Station on the influence of larval trematode infections in lymnaeid snails on their second intermediate host relations to the strigeid trematode, *Cotylurus flabelliformis*. Whenever the cercariae of *C. flabelliformis* are able to get into the germinal sacs of trematodes parasitic in snails the metacercariae develop normally to the encysted stage as hyperparasites. In fact, they develop more rapidly in this location than in their normal habitat, the hermaphroditic gland. In snails infected with the sporocysts of *C. flabelliformis* there is a specific immunity produced against the cercariae of this species which prevents all but a few from penetrating. In other words, snails serving as intermediate hosts for this trematode species cannot serve effectively as second intermediate hosts for the development of its metacercariae. In normal second intermediate hosts the presence of the germinal sacs of certain species of larval trematodes does not affect the numbers of metacercariae that develop. Other species, however, produce in the infected snails a nonspecific immunity which greatly reduces the numbers of metacercariae that develop. Both the specific and the nonspecific immunity in the snails infected with these larval trematodes appear to be caused by some change in the snails that prevents most of the cercariae from penetrating. The few that do get in find their way into the germinal sacs of the trematodes and develop normally.

In snails that are abnormal second intermediate hosts for *C. flabelliformis* the presence of the germinal sacs of larval trematodes tends to break down the resistance

and allows numbers of the metacercariae to develop. In completely abnormal hosts such as physid and planorbid snails the cercariae penetrate but fail to develop unless trematode parasites are present. In the infected snails they penetrate into the germinal sacs and there develop normally. The same relation was found to be true in *L. stagnalis* and *S. emarginata* which are abnormal second intermediate hosts for the varieties of *C. flabelliformis* the cercariae of which develop in *S. emarginata* and *L. stagnalis* respectively. Almost no development of metacercariae takes place in *L. stagnalis* when exposed to infection with the cercariae of *C. flabelliformis* from *S. emarginata*. In snails infected with the sporocysts of *D. flexicaudum*, however, the cercariae of this variety develop in large numbers. Also, almost no development of metacercariae takes place in juveniles of *S. emarginata* when exposed to infection with the cercariae of *C. flabelliformis* from *L. stagnalis*. When, however, the juveniles of this species harbor the sporocysts of certain species of larval trematodes the metacercariae of this variety develop in large numbers. Adults of *S. emarginata*, while much more susceptible than juveniles to the variety of the cercariae of *C. flabelliformis* from *L. stagnalis* still show a degree of abnormality. Infection with certain species of larval trematodes breaks this partial resistance and permits much larger numbers of the metacercariae to develop than in uninfected snails, while the presence of certain other species increases this resistance so that the snails are almost completely immune to the development of the metacercariae. Perhaps most surprising of all was the finding that infections with certain species of larval trematodes (*D. flexicaudum* and *P. muris*) produce nonspecific immunity in normal second intermediate hosts and also increase the susceptibility of abnormal hosts.

## REFERENCES

- BEAVER, P. C. 1937 Experimental studies on *Echinostoma revolutum* (Froelich) a fluke from birds and mammals. Illinois Biol. Monog. 25(1): 1-96.
- CORT, W. W. 1917 Homologies of the excretory system of the forked-tailed cercariae. A preliminary report. J. Parasitol. 4: 49-57.
- AND BRACKETT, STERLING 1937a Precocious development of the metacercaria stage of *Diplostomum flexicaudum* in the snail intermediate host. J. Parasitol. 23: 245-246.
- AND — 1937b Two new species of strigeid cercariae from the Douglas Lake region, Michigan. J. Parasitol. 23: 265-280.
- AND — 1938 Two new species of strigeid cercariae in *Stagnicola palustris elodes* (Say) from the Douglas Lake region, Michigan. Tr. Am. Micr. Soc. 57: 274-281.
- , — AND OLIVIER, LOUIS 1944 Lymnaeid snails as second intermediate hosts of the strigeid trematode, *Cotylurus flabelliformis* (Faust, 1917). J. Parasitol. 30: 309-321.
- AND BROOKS, S. T. 1928 Studies on the holostome cercariae from Douglas Lake, Michigan. Tr. Am. Micr. Soc. 47: 179-221.
- , McMULLEN, D. B. AND BRACKETT, STERLING 1937 Ecological studies on the cercariae in *Stagnicola emarginata angulata* (Sowerby) in the Douglas Lake region, Michigan. J. Parasitol. 23: 504-532.
- , OLIVIER, LOUIS AND BRACKETT, STERLING 1941 The relation of physid and planorbid snails to the life cycle of the strigeid trematode, *Cotylurus flabelliformis* (Faust, 1917). J. Parasitol. 27: 437-448.
- McMULLEN, D. B. 1937 The life histories of three trematodes, parasitic in birds and mammals belonging to the genus *Plagiorchis*. J. Parasitol. 23: 235-243.
- 1938a Observations on precocious metacercarial development in the trematode superfamily Plagiorchioidea. J. Parasitol. 24: 273-280.
- 1938b Notes on the morphology and life cycles of four North American cercariae. Livro Jubilar Prof. Travassos, 299-306.
- NOLF, L. O. AND CORT, W. W. 1933 On immunity reactions of snails to the penetration of the cercariae of the strigeid trematode, *Cotylurus flabelliformis* (Faust). J. Parasitol. 20: 38-48.

- OLIVIER, LOUIS AND CORT, W. W. 1941a *Cercaria douglasi* Cort, 1917, and its relation to the cercaria of *Cotylurus flabelliformis* (Faust, 1917). J. Parasitol. 27: 343-346.
- AND ——— 1941b An experimental test of the life cycle described for *Cotylurus communis* (Hughes). J. Parasitol. 28: 75-81.
- PRICE, HELEN F. 1931 The life history of *Schistosomatium douthitti* (Cort). Am. J. Hyg. 13: 685-727.
- TALBOT, S. B. 1936 Studies on Schistosome dermatitis II. Morphological and life history studies on three dermatitis producing schistosome cercariae, *C. elvae* Miller, 1923, *C. stagnicola* n. sp., and *C. physellae* n. sp. Am. J. Hyg. 23: 372-384.
- VAN HAITSMA, J. P. 1931a Studies on the trematode family Strigeidae (Holostomidae) No. XXII. *Cotylurus flabelliformis* (Faust) and its life history. Papers Michigan Acad. Sc., Arts, and Letters 13: 447-482.
- 1931b Studies on the trematode family Strigeidae (Holostomidae) No. XXIII. *Diplostomum flexicaudum* (Cort and Brooks) and stages in its life history. Papers Michigan Acad. Sc., Arts, and Letters 13: 483-516.
- WINFIELD, G. F. 1932 On immunity of snails infested with the sporocysts of the strigeid, *Cotylurus flabelliformis*, to the penetration of its cercariae. J. Parasitol. 19: 130-133.

## INGESTION PROCESSES ON *IODAMOEBEA* (PROTOZOA)

ROBERT M. STABLER

Department of Zoölogy, University of Pennsylvania

In discussing the food habits of *Entamoeba muris* and *E. ranarum*, Wenrich (1941) described the presence of differentiated "pharynges" and tubes which appeared to be used in ingestion. In 1943 he described similar structures on a flagellate, *Histomonas meleagridis*, from domestic chickens and pheasants (*Phasianus torquatus*). He recently (1944) noted them on *Dientamoeba fragilis* from man.

The following observations were made on *Iodamoeba* from man and two other primates. These structures were not noted by Wenrich (1937) in his paper on *Iodamoeba bütschlii*.

### MATERIAL

The present studies concerned two cases of *Iodamoeba bütschlii* infection in man, and one of *Iodamoeba kueneni* from the chimpanzee. Brief observations were also made on a similar infection in a Guinea baboon, from the Philadelphia Zoölogical Gardens.

The material was fixed in Schaudinn's fluid plus 5 per cent of glacial acetic acid, and stained in Heidenhain's hematoxylin.

Thanks are due Mr. Warren E. Buck of Camden, New Jersey, for the chimpanzee material, and to Dr. D. H. Wenrich who made the slides from this host.

### OBSERVATIONS

The tube-like structures found on these organisms varied considerably in size and general morphology. A single amoeba frequently showed those of more than one type. As many as four of these processes were occasionally seen on one organism. Some were quite large (Fig. 1), while others were small. Many were twisted (Figs. 1; 3; 7), others were straight (Figs. 2; 5). There were food objects in some projections (Figs. 1; 2; 5; and lower process on 6), while others were apparently about to engulf them (Figs. 1; 8).

In many projections the walls were not especially darkly stained (Figs. 1; 2; upper process on 4; 5; 8). These appeared to be the more recently formed. In general, those with darkened walls (Figs. 3; lower process on 4; 6; 7) appeared to be contracting or in the process of being resorbed. The lower projection on the amoeba shown in Fig. 6, then, might be a later stage than that figured on 5, where, after the original contact with the food (5), the projection has withdrawn toward the body (6), resulting in more deeply staining walls. The upper left process on Fig. 6 probably represents the last stage in resorption.

There is also some evidence that ingestion may be accomplished by the formation of food cups on the surface of the amoeba.

Counts were made to determine the frequency of these projections. In one case, 300 trophic forms of *Iodamoeba bütschlii* included 35 with processes (11.7 per cent), while in another, 135 out of 500 showed them (27.0 per cent). In 100 individuals of *I. kueneni* (chimpanzee) examined at random, only one possessed a tube (Fig. 8).



Though showing occasional projections, the material from the Guinea baboon was unsuitable for statistical analysis.

#### DISCUSSION

The processes herein described on *Iodamoeba* appear to function in an ingestatory capacity. Some would seem to be tube-like, at least in part. They are similar to structures described by Wenrich on *Entamoeba muris* and *E. ranarum* (1941), on *Histomonas meleagridis* (1943), and on *Dientamoeba fragilis* (1944). They are more like those from the amoebae, however, in lacking the dense, deeply-staining core surrounded by a layer of clear ectoplasm, as described in the projections from the flagellate, *Histomonas*. They more nearly resemble the structures on *Histomonas* in the degree to which they may extend from the body proper. For further discussion of function, mode of operation, etc., see the papers by Wenrich.

#### SUMMARY

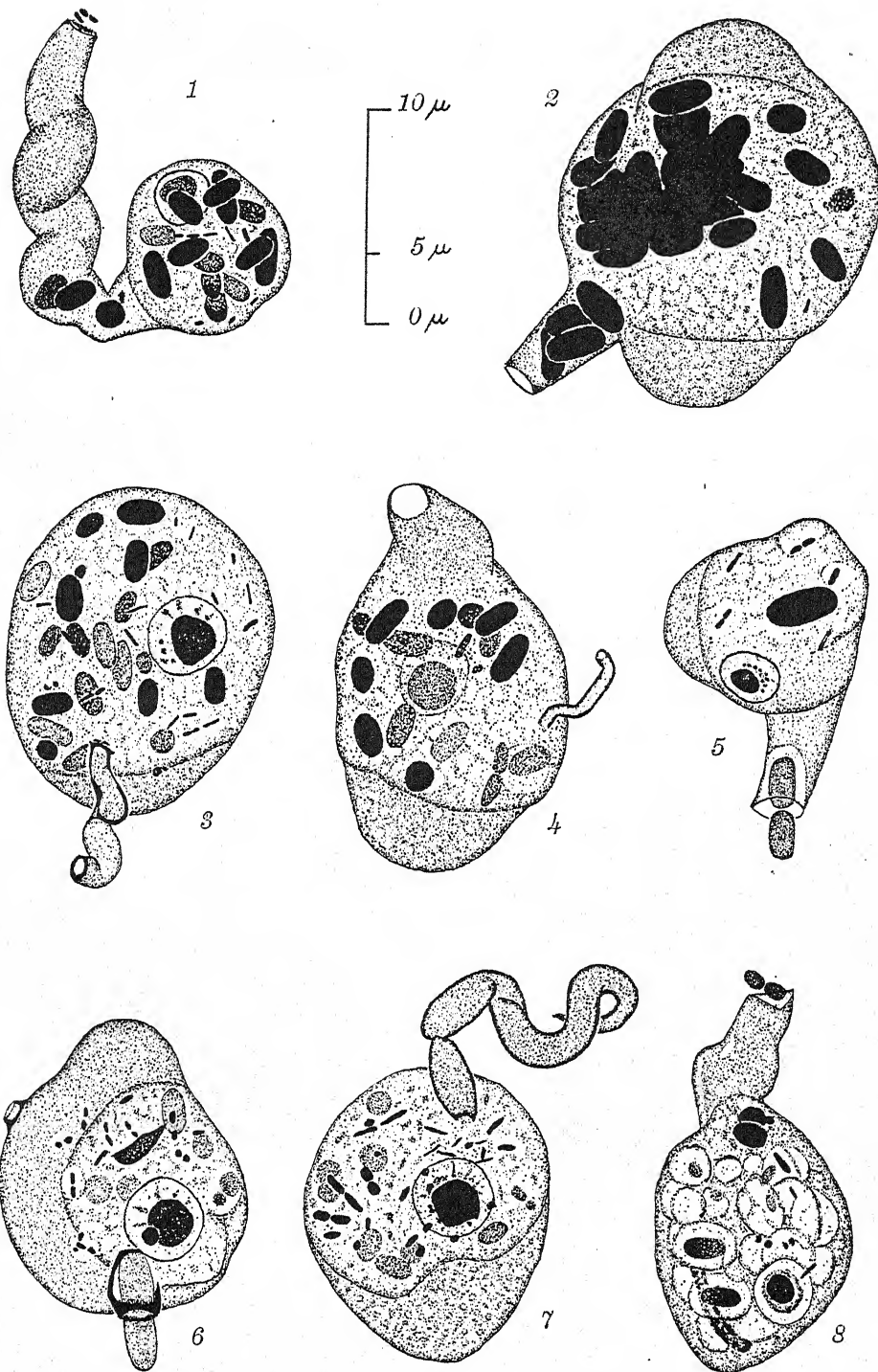
1. Both *Iodamoeba bütschlii* from man and *I. kueneni* from the chimpanzee and Guinea baboon may produce tube-like projections some of which, at least, appear to be acting as organs of ingestion.
2. In two cases from man the frequency of the processes was 11.7 and 27.0 per cent respectively.

#### REFERENCES

- WENRICH, D. H. 1936 Studies on *Iodamoeba bütschlii* (Protozoa) with special reference to nuclear structure. *Proc. Amer. Phil. Soc.* 77: 183-205.
- 1941 Observations on the food habits of *Entamoeba muris* and *Entamoeba ranarum*. *Biol. Bull.* 81: 324-340.
- 1943 Observations on the morphology of *Histomonas* (Protozoa, Mastigophora) from pheasants and chickens. *J. Morph.* 72: 279-303.
- 1944 Studies on *Dientamoeba fragilis* (Protozoa). IV. Further observations, with an outline of present-day knowledge of this species. *J. Parasitol.* 30: 322-338.

#### DESCRIPTION OF THE PLATE

- Figures 1-7 are of *Iodamoeba bütschlii* from man; fig. 8 is *I. kueneni* from the chimpanzee.
- FIG. 1. Long, twisted projection, with food objects at either end.
- FIG. 2. Amoeba with short projection containing food.
- FIG. 3. Note twisted projection with deeply stained areas in the walls, suggesting retraction.
- FIG. 4. Upper projection appears freshly formed and tube-like at end; lower one retracting(?).
- FIG. 5. Bacteria in the act of entering the process.
- FIG. 6. Upper left projection appears in the last stages of resorption; lower one ingesting, and also withdrawing(?).
- FIG. 7. Long, twisted process, thick-walled and retracting(?).
- FIG. 8. Process appearing to be at the moment of ingestion. (Slide courtesy of Dr. D. H. Wenrich.)



## RESEARCH NOTE

### AN INSTANCE OF PHAGOCYTOSIS OF *TRICHOMONAS FOETUS* IN BOVINE VAGINAL SECRETIONS

Wenrich and Emmerson (1933, J. Morph. 55: 193-205) reported that in stained preparations of vaginal discharge from a cow infected with *Trichomonas foetus* many of the leucocytes contained ingested cellular elements, which in some cases could be identified as ingested flagellates. They suggested that this indicated an activity on the part of the leucocytes in defense of the host. An observation by the present authors, made in the course of daily observations on heifers and cows during the course of their infections with *T. foetus*, supports this viewpoint.

The vaginal sample on which our observation was made was obtained from a heifer undergoing her initial infection; she was bred as a virgin, at the age of 19 months, to an infected bull. Trichomonads were first detected in vaginal samples on the sixth day following coitus. The trichomonads rapidly increased and were present in large numbers until the 26th day following coitus. On that day the numbers suddenly decreased, and on the next day the heifer returned to estrum. Following this, no trichomonads could be demonstrated in the vaginal samples until the 41st day after coitus, except that one day (33rd), a very small number of trichomonads were observed. On the 41st day, large numbers of trichomonads appeared (200,000 per cc of vaginal sample estimated by hemacytometer count); on the 42nd day the number was still greater (2,750,000 per cc). In samples collected on both of these days the trichomonads were moving slowly, in contrast to the high level of activity shown by the organisms before the return to estrum. On the 43rd day the trichomonads were present in much lower numbers (6,000 per cc) and most of them were stationary. In making the count of trichomonads from this sample with the aid of a hemacytometer, a leucocyte containing an actively moving body within a large vacuole was seen. This body, resembling *T. foetus*, performed rotating movements for the period of about a half-hour that it was under observation. Many other leucocytes had similar large vacuoles, but these appeared to be empty of formed matter. There were intermediate stages between these leucocytes with large vacuoles and typical leucocytes in which there were no apparent vacuoles. We believe that the actively moving body within the leucocyte was a trichomonad in process of being phagocytized. On the following six days, until the next estrum, the samples contained widely fluctuating numbers of trichomonads. It appears likely that at this stage of the infection the activity of the leucocytes was a factor in reducing the numbers of trichomonads in the vagina.—DATUS M. HAMMOND AND DAVID E. BARTLETT, *Zoological Division, Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville Research Center, Beltsville, Md.*

---

Mailing dates for Volume 30 (1944) were:

- No. 1, February 15.
- No. 2, May 22.
- No. 3, (with August Supplement), August 2.
- No. 4, October 3.
- No. 5, November 10.
- No. 6, January 17, 1945.

# The Journal of Parasitology

Volume 31

APRIL, 1945

Number 2

## SIPHONAPTERA: THE GENUS *OROPSYLLA* IN NORTH AMERICA\*

WM. L. JELLISON

Sanitarian (R), United States Public Health Service

Fleas of the genus *Oropsylla* are characteristic ectoparasites of several species of fossorial rodents in the genera *Citellus* and *Marmota* of the family SCIURIDAE in North America and Asia. As *Oropsylla rupestris* (Jordan) and, to a lesser extent, *O. idahoensis* (Baker) have been found by Eskey and Haas (1940) to be experimental vectors of plague, and as their natural hosts have been found infected with plague in nature in many Western States and Canada, a taxonomic review of the genus *Oropsylla* seems appropriate.

*Oropsylla* Wagner and Ioff 1926

*Male and Female:* Typical ceratophylline fleas of medium to large size and of golden brown color. Eye prominent, gena rounded, not acute, frontal tubercle small (very small in *O. alaskensis*), maxillae long and acute, labial palpi large and long extending beyond the trochanters. A few small bristles present on the outside of the forefemur, inside of hind coxae with very numerous long fine bristles near anterior margin from base to apex. Five pairs lateral plantar bristles on the fifth segment of each tarsus though odd specimens may have 6 in some rows, proximal pair of plantar bristles not displaced inward. Well developed pronotal ctenidium, apical spinelets on anterior abdominal tergites and sometimes on metanotum. Basal abdominal sternite with ventral bristles but lacking a patch of lateral bristles except in ♀ of *O. alaskensis* which has 4 or 5. Some abdominal tergites with 3 definite rows of bristles and additional scattered bristles. Lower bristles on most abdominal tergites below the level of the row of spiracles.

*Female:* Sternite VII entire without prominent lobes or incision, head of spermatheca ovate, slightly longer than broad, tail about as long as head and may be expanded in *O. arctomys* and *O. rupestris*, and in all species has prominent terminal appendage (a most typical character of the genus). Three and often 4 large antepygidial bristles on each side, stylet with 2 to 4 lateral and a long terminal bristle.

*Male:* Sternite VIII long and narrow with a terminal or subterminal group of 4 to 6 long bristles but without a membranous appendage. One large, one minute antepygidial bristle. Process and finger of the clasper broad with numerous bristles but no black spines. Levers of penis with about one complete convolution.

The original description of the genus by Wagner and Ioff (1926) is quoted:

Der massive Rüssel ist stark verlängert; das ganze letzte Gliedchen der Unterlippentaster überragt den Trochanter; dies Gliedchen ist nicht weniger als doppeltlang im Vergleich zu dem vorletzten Gliedchen und länger als das 3. und 4. zusammen. Die frontale Reihe ist nicht vollkommen entwickelt, es sind davon bloß die Eckborsten erhalten (gewöhnlich 2) so wie die oberen—bis auf 3 Härchen—recht schwache und weit gegen den Rücken fortgeschobene. Die Scheitelborstenreihen—rudimentär. Die Antennenkeule ist bei dem ♀ von einer etwas verlängerten ovalen Form, bei dem ♂ aber ist sie stark verlängert. Die Anzahl der Antepygidialborsten ist beim ♂—1, bei der ♀—3. Das achte Abdominalsternit der ♀ ist halbrudimentär, kurz, mit langen Borsten. Receptalum seminis—mit einem Chitinauswuchs, am Ende seiner engen Distalabteilung, sein Reservoir (Proximalabteilung) von ovaler Form.

Received for publication, October 16, 1944.

\* Contribution from Rocky Mountain Laboratory, (Hamilton, Montana), Division of Infectious Diseases, National Institute of Health.

Collection data of *Oropsylla arctomys* (Baker)

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
18079	Alaska	Fairbanks	7-17-1937	<i>M. monax ochracea</i>	4♂, 6♀	C. B. Philip
	British Columbia	Eagle River (North Fork)	5-22-1938	<i>M. caligata okanagana</i>	1♂	G. P. Holland
	"	Quick	6-5-1943	<i>Gnath. latrans</i> (cayote)	2♂, 2♀	"
	"	Wigwam Mine	5-21-1939	<i>M. monax petrensis</i>	2♂, 1♀	"
	Saskatchewan	Vavenny	8-17-1931	"	5♂, 3♀	"
	"	Big River	8-15-1942	<i>Marmota</i> sp.	1♂	"
	"	Kendal	9-18-1943	Man	2♂, 3♀	"
	Canada	Ontario	10-1-1930	<i>Marmota</i> sp.	1♂	U.S. Nat. Museum
6085	Montana	Ravalli Co.	4-25-1930	<i>M. flaviventris</i>	1♂	Rocky Mountain Laboratory
14537	Wyoming	Albany Co.	6-15-1938	"	1♂	U.S. Nat. Museum
	Minnesota	Cloquet	4-9-1936	<i>M. monax</i>	1♂	Univ. Michigan
	"	Berrien Co.	7-11-1919	"	1♂	G. W. Luttermoser
	"	Cheboygan Co.	July 1935	"	2♂, 1♀	Univ. Michigan
15828	"	Goehle Co.	6-30-1920	"	1♂, 1♀	"
15836	"	"	Aug. 1920	"	3♂, 1♀	"
18077	"	"	8-11-1920	<i>Mephitis</i> sp. (3 hosts)	1♂, 2♀	"
15945	"	"	8-11-1920	"	1♂, 1♀	"
15878	"	"	Oct. 1940	"	3♂, 3♀	"
15881	"	Lincoln, Penobscot Co.	5-20-1918	<i>M. monax</i>	1♂, 1♀	W. Clayton through Robt. Traub
15883	"	Horseshoe Island	7-17-1926	"	1♂	L. C. Stegeman
18250	"	Keeseville, Essex Co.	7-9-1900	"	6♂, 9♀	U.S. Nat. Museum
16503	"	North Elba	"	"	2♂, 2♀	"
	"	Peterboro	"	"	1♂	"
	"	Rockland Co.	"	"	1♂, 1♀	"
	"	Syracuse, Onondago Co.	"	"	6♂, 9♀	"
	"	Monroe	"	"	2♂, 2♀	"
17403	Connecticut	Ablington	4-12-1924	<i>Mephitis</i> sp.	1♂	L. C. Stegeman
16226	Massachusetts	Belmont	7-8-1926	<i>M. monax</i>	1♂	U.S. Nat. Museum
	"	Charles River Village	6-13-1938	"	1♂	"
	"	"	1-11-1923	<i>Mephitis</i> sp.	1♂	"
	"	Holbrook	3-13-1924	<i>M. monax</i>	1♂, 1♀	"
	"	Needham, Norfolk Co.	3-26-1927	"	1♂, 1♀	"
	"	Newton Center	9-20-1926	"	3♂, 1♀	"
	"	Sagamore	7-29-1934	"	1♂, 1♀	"
	"	Canobie Lake	July 1892	"	1♂, 1♀	"
	New Hampshire	"	Mar. 1901	<i>Mustela</i> (mink)	1♂	"
	"	Concord	5-15-1926	<i>Vulpes</i> (red fox)	1♀	"



Collection data of *Oropsylla rupestris* (Jordan)

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
8821	Alberta	Calgary	6-28-1940	<i>C. richardsoni</i>	2♂, 2♀	G. P. Holland (topotypes)
	"	Edmonton	8-28-1932	<i>Citellus</i> sp.	1♂, 6♀	Rocky Mountain Laboratory
	"	High River	7-28-1938	<i>C. richardsoni</i>	1♂	G. P. Holland
	Saskatchewan	Estevan	8-6-1942	<i>Mustela</i> sp.	1♂	"
	"	Rock Glen	9-9-1942	<i>C. richardsoni</i>	1♂, 1♀	"
	"	Saskatoon	6-6-1941	"	2♂	"
18777	"	"	6-6-1941	"	1♂, 3♀	L. G. Saunders
	"	"	10-10-1940	<i>Mustela</i> sp.	1♂	G. P. Holland
18105	Montana	Val Marie	7-8-1942	"	1♂, 1♀	Rocky Mountain Laboratory
18199	"	Blaine Co.	5-25-1937	<i>C. richardsoni</i>	1♂, 1♀	Montana State College
10054	"	Gallatin Co.	3-11-1929	"	3♂	Rocky Mountain Laboratory
	"	"	3-15-1929	"	1♂	C. P. Haight, Montana State College
	"	"	4-7-1940	"	1♂, 1♀	"
	"	"	March 1940	"	1♂, 1♀	"
	"	Glacier Co.	6-14-1940	"	1♂, 1♀	"
18967	"	Wheatland Co.	8-27-1935	"	1♂, 1♀	E. C. Cates, Montana State College
19099	N. Dakota	Barnes Co.	8-10-1941	"	8♂, 9♀	Rocky Mountain Laboratory
18992	"	Benson Co.	8-28-1941	"	2♂, 1♀	"
19088	"	Mellenville Co.	8-20-1941	"	3♂, 1♀	"
	"	"	8-25-1941	<i>Citellus</i> sp.	7♂, 6♀	"
19117	"	Pierce Co.	9-2-1941	<i>C. richardsoni</i>	5♂, 3♀	"

Collection data of *Oropsylla idahoensis* (Baker)

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
18241	Alaska	Cantwell	7-10-1939	<i>Citellus</i> sp. (several hosts)	9♂, 17♀	C. B. Philip
17280	"	Little Delta River	4-14-1940	"	1♂, 3♀	J. Warwick
	"	and Shumagin Island				
18239	"	Rapids	July 1880	" = " <i>Spermophilus</i> "	3♂, 2♀	U.S. Nat. Museum by F. H. Bean
17281	British Columbia	Richardson Hiway	7-15-1939	" (several hosts)	2♂, 2♀	C. B. Philip
	"	Isaac Creek	9-12-1941	"	2♂, 4♀	J. Warwick
	"	Mt. Begbie	8-9-1939	<i>C. columbianus</i>	2♂, 2♀	G. P. Holland
	Canada	Jasper National Park	8-2-1941	"	1♂, 1♀	"
	Alaska	Panik	7-2-1940	"	1♂, 1♀	"
	"		6-5-1943	<i>C. lateralis</i>	5♂, 1♀	"
	Washington	Cofax, Whitman Co.	7-2-1939	<i>C. columbianus</i>	2♂, 2♀	U.S. Nat. Museum
8220A	"	Iron Springs	4-21-1927	<i>Thomomys fuscus</i>	1♀	"
8250A	Oregon	Hurney Co.	5-23-1930	<i>C. columbianus</i>	1♂	Rocky Mountain Laboratory
	"		7-17-1932	<i>C. lateralis</i>	1♂	"
	"		7-18-1923	<i>C. t. mollis</i>	2♂, 2♀	"
9180B	"	Keno, Klamath Co.	8-10-1934	<i>C. columbianus</i>	1♂, 7♀	U.S. Nat. Museum
13177	"	Lake Co.	9-20-1933	<i>Citellus</i> sp. (2 hosts)	1♂, 4♀	Rocky Mountain Laboratory
18417	Idaho	Arco, Butte Co.	5-8-1937	<i>C. armatus</i>	5♂, 5♀	" (topotypes)
12707	"	Moscow Mts., Latah Co.	6-6-1936	<i>C. columbianus</i>	2♂, 5♀	"
18244	"	Beaverhead Co.	7-28-1936	<i>C. lateralis</i>	1♂	"
18246	"	"	6-21-1937	<i>Asio wilsonianus</i> , owl		"
	"	"	June &			"
18358	"	"	July 1937	<i>C. armatus</i> (several hosts)	3♀	"
14128	"	"	7-19-1937	<i>Pica pica hudsonia</i> , magpie	1♂	"
14182	"	"	July 1938	<i>C. columbianus</i> (several hosts)	8♀	"
	"	"	July 1938	<i>Citellus</i> sp., probably <i>r. elegans</i>	2♂, 10♀	"
	"	"	(4 other collections this host and county.)			"
	"	"	6-27-1936	Nest of <i>Buteo</i> , hawk	1♂, 1♀	"
	"	"	7-6-1936	"	1♀	"
18698	"	Flathead Co.	5-1-1941	<i>C. columbianus</i>	2♀	C. P. Haight, Montana State College
	"	Granite Co.	5-17-1940	"	1♂, 4♀	"
12671	"	Lake Co.	6-11-1940	"	1♀	Rocky Mountain Laboratory
12669	"	Madison Co.	6-15-1940	<i>C. armatus</i>	3♂, 4♀	"
	"	"	6-16-1936	<i>Citellus</i> sp. (5 hosts)	5♂, 7♀	"
	"	"	June 1936	<i>C. armatus</i>	1♀	C. P. Haight, Montana State College
	"	"	7-24-1940	<i>C. columbianus</i>	3♂, 9♀	"
2364	"	Missoula Co.	May 1940	<i>C. lateralis</i>	1♂, 4♀	Rocky Mountain Laboratory
14076	"	Ravalli Co.	4-17-1923	"	3♂, 10♀	"
	"	"	(Numerous other collections from same host and locality.)			"
	"	"	(Numerous other collections from same host and locality.)			"
14414	Wyoming	Silver Bow Co.	July 1938	<i>C. columbianus</i> (3 hosts)	1♀	C. P. Haight
14449	"	Albany Co.	7-16-1940	"	5♀	Rocky Mountain Laboratory
14450	"	"	5-24-1938	<i>C. r. elegans</i>	1♂, 1♀	"
14417	"	"	May 1938	<i>C. lateralis</i>	3♂, 3♀	"
	"	"	5-14-1938	" (3 hosts)	2♀	"
	"	Natrona Co.	8-17-1925	<i>Citellus</i> sp.	1♂	U.S. Nat. Museum
	"	Yellowstone Park	7-26-1929	<i>C. lateralis</i>	1♂, 1♀	"
	Utah	Salt Lake	8-2-1929	<i>C. armatus</i>	2♂, 1♀	"
	"	Strawberry Valley	6-1-1942	"		"

Collection data of *Oropsylla idahoensis* (Baker)—Continued

Accession No.	State or country	Locality	Date	Host animal or source	Number	Collector or authority
18117	California	San Bernardino Co.	5-4-1936	<i>C. lateralis</i>	1 ♀	Rocky Mountain Laboratory
8951	Colorado	Archuleta Co.	6-11-1932	"	3 ♂, 1 ♀	"
1644	"	Boulder Co.	April, 1	" (many hosts)	70 ♂, 76 ♀	"
and other numbers	"		May, June, July, 1940	"		
8008A	"	Conchos Co.	8-11-1931	"	1 ♂, 1 ♀	Montana State College
15692	"	"	(Numerous specimens same locality and host.)	"		
15665	"	Gilpin Co.	7-11-1932	<i>Cynomys</i> sp.	2 ♀	Rocky Mountain Laboratory
and other numbers	"	Lake Co.	6-26-1939	<i>C. lateralis</i>	3 ♂, 3 ♀	"
15344	"	"	6-22-1939	<i>Citellus</i> sp. (5 hosts)	4 ♂, 7 ♀	"
15710	"	Larimer Co.	2-23-1939	"	2 ♂	"
15703	"	Moffat Co.	6-6-1938	<i>C. r. elegans</i>	1 ♂	"
and other numbers	"	Park Co.	6-30-1939	<i>Citellus</i> sp.	1 ♂, 2 ♀	"
13729	"	"	6-30-1939	<i>Cynomys</i> sp. (4 hosts)	16 ♂, 82 ♀	"
15663	"	Pike Co.	7-25-1937	<i>C. lateralis</i>	5 ♂, 1 ♀	"
and other numbers	"	Summit Co.	6-22-1939	<i>Citellus</i> sp. (4 hosts)	6 ♂, 8 ♀	"
16925	"	Teller Co.	8-6-1924	<i>C. lateralis</i> (2 hosts)	2 ♂, 1 ♀	"
11000D	Arizona	Coconino Co.	5-21-1935	" (3 hosts)	2 ♂, 10 ♀	"
and other numbers						

Wagner and Ioff (1926) described the genus *Oropsylla* in Russian, and also in German as quoted above, naming as the genotype *Ceratophyllus silantiewi* Wagner 1898, a parasite of Asiatic marmots, *Marmota bobac* and *M. siberica*. They also included in the genus a new species, *O. ilovaiskii*, a species described by Jordan and Rothschild (1911) as *C. crassus* from *Marmota robusta* in China and "several others." Wagner (1930) considered *O. crassus* as a subspecies of *O. silantiewi* and added *C. elana* Jordan 1929 to the genus. He also tentatively referred *C. mandarinus* to *Oropsylla*.

In a discussion of the American species of fleas on ground squirrels and marmots, Wagner (1929) referred six species to the genus *Oropsylla*, namely, *C. alaskensis* Baker 1904, *C. bruneri* Baker 1895, *C. idahoensis* Baker 1904, *C. montanus* (Baker) 1895, *C. rupestris* Jordan 1925, and *C. tuberculatus* Baker 1904. Jordan (1933) reviewed the American species of the genus *Ceratophyllus* (*sensu lato*) and recognized or described a total of 18 genera. In this review, the genus *Oropsylla* was redefined and the American species were restricted to *O. alaskensis*, *O. arctomys*, *O. idahoensis* and *O. rupestris*. He also assigned the Asiatic species *mandarinus*, which Wagner had tentatively referred to *Oropsylla*, to the new genus *Diamanus*. Ewing and Fox (1943), in a treatise on the North American fleas, placed *Diamanus* Jordan and *Opisocrostis* Jordan as subgenera of *Oropsylla*, an arrangement with which the writer does not agree.

*Oropsylla arctomys* (Baker) 1904 = *Aetheopsylla septentrionalis* Stewart and Holland 1940

1929. *Oropsylla arctomys* (Baker). Jordan, Nov. Zool. 35: 32.  
 1936. *Oropsylla arctomys* (Baker). Ioff, Zeit. Parasit. 9: 73-124.  
 1939. *Oropsylla arctomys* (Baker). Jellison and Good, Nat. Inst. of Health Bull. No. 178.  
 (Bibliography of species up to 1939.)  
 1939. *Oropsylla arctomys* (Baker). Jellison and Kohls, Pub. Health Rept. 54: 2022.  
 1940. *Oropsylla arctomys* (Baker). Eskey and Haas, Pub. Health Rept. 254: 73.  
 1940. *Oropsylla arctomys* (Baker). Fox, Fleas of Eastern United States, pp. 45-46.  
 1940. *Aetheopsylla septentrionalis* Stewart and Holland, Can. Ent. 72: 41-42.  
 1942. *Oropsylla arctomys* (Baker). Fuller, Ent. News 53: 137.  
 1943. *Oropsylla* (*Oropsylla*) *arctomys* (Baker). Ewing and Fox, U. S. Dept. Agric. Misc. Pub. No. 500: 49.  
 1943. *Oropsylla arctomys* (Baker). Fuller, Jour. New York Ent. Soc. 51: 5.  
 1943. *Oropsylla arctomys* (Baker). Fuller, Bull. Brook. Ent. Soc. 38: 20.  
 1943. *Oropsylla arctomys* ———. Bell and Chalgren, Jour. Wildlife Management 7: 275.

*Male*: Length 2.9 mm,<sup>1</sup> frontal tubercle prominent in some specimens, much reduced in others, labial palps equaling forefemur, preoral bristles 1 or 2 pairs, 1 large and numerous small bristles along dorsal border of antennal groove; pronotal ctenidium of 16 to 18 spines in specimens from eastern United States, and 20 to 22 spines in Alaskan specimens; apical spinelets on each side as follows: metanotum 1, abdominal tergites I to III, 2-1-1 respectively; 1 large and 1 very small antepygial bristle on each side. Only one acetabular bristle on each side. This character was mentioned in the original description. It is found to hold in the long series available for study and in most specimens of the closely related species, *O. rupestris*. Moveable process of the clasper broadly club-shaped, convex on anterior margin; sternite VIII with 4 large terminal and 3 or 4 pairs of smaller ventral bristles; 5 pairs of lateral plantar bristles on each terminal tarsal segment but occasional specimens have 6 pairs.

*Female*: Length 3.6 mm, frontal tubercle less distinct than in the male, row of bristles on second segment of antennae exceeding the club; apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 1-2-2-2 respectively with some variation in different speci-

<sup>1</sup> There is a great variation in size of fleas of the same species. This and other measurements are from representative specimens and are not averages or means. They may disagree with published descriptions.

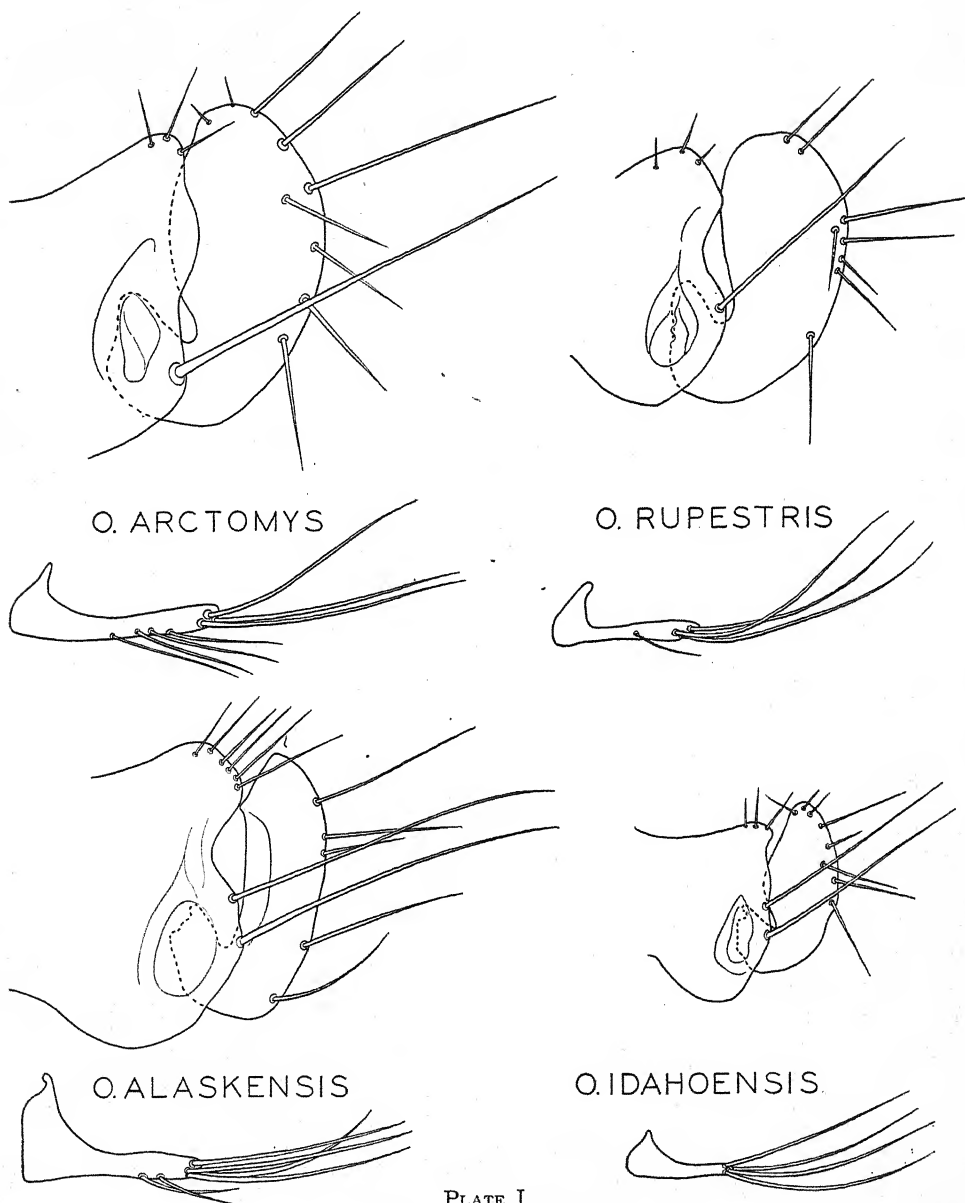


PLATE I

Claspers and Sternites VIII of Males.

SPECIMENS FIGURED IN PLATES I AND II.

*Oropsylla arctomys* (Baker):♂ from *Marmota monax*, Horseshoe Island, New York, June 4, 1932.♀ from *Marmota monax*, Gogebic Co., Michigan, June 30, 1920.*Oropsylla rupestris* (Jordan):♂ and ♀ from *Citellus richardsoni*, Calgary, Alberta, June 28, 1940. (Topotypes)*Oropsylla alaskensis* (Baker):♂ and ♀ from *Citellus parryi*, Thelon Game Preserve, Northwest Territories, Canada, June 24, 1937.*Oropsylla idahoensis* (Baker):♂ and ♀ from *Citellus columbianus*, Moscow, Idaho, June 1936. (Topotypes)

(The figures indicate a close relationship rather than striking differences in the four American species.)



mens, 3 antepygidial bristles on each side in most specimens but some with 4, stylet about  $1\frac{1}{2}$  times longer than wide with a large terminal, and 3 lateral bristles, sternite VII rounded on dorsal posterior corner except in Alaskan specimens and with a row of 8-10 large bristles and 5-10 smaller bristles anteriorly, Alaskan specimens with very numerous bristles; receptaculum figured.

*Oropsylla arctomys* was described by Baker from specimens taken from the eastern woodchuck, *Marmota monax*, "*Arctomys monax*," at Peterboro, New York, July 9, 1900. *M. monax* is the normal host of this species and it is apparently the only flea found regularly on this host, judging by published records and numerous collections examined by the writer. Its distribution probably coincides with the distribution of the several subspecies of *M. monax* from Massachusetts and New York across northern central United States, through Michigan, Minnesota to British Columbia and north to Alaska. The series of specimens from Alaska was collected by Dr. C. B. Philip from *Marmota monax ochracea*, near Fairbanks. This flea is present but extremely rare in the Rocky Mountain region of the Western States in the range of *Marmota flaviventris*, the western woodchuck, and south of the range of *M. monax* which extends only into a small area of northern Montana and Idaho. These western woodchucks are consistently and abundantly infested with fleas of the genus *Thrassis*, including *T. acamantis*, *T. howelli* and several other closely related species or subspecies. These outnumber *O. arctomys* on *M. flaviventris* 1000-1. Collections of fleas from *M. monax* that have been examined usually contain from 1-10 specimens, while *M. flaviventris* is often found infested with 25 to 50 or more specimens of *Thrassis*. Eskey and Haas (1940) give averages of 13, 22 and 56 fleas per marmot in three western areas. No species of *Thrassis* is found on *M. monax* in eastern North America, nor on Asiatic marmots which are infested with *Oropsylla*. Within the range of *M. monax*, *O. arctomys* is also a common parasite of skunks, *Mephitis* spp., and other carnivores, which indicates predation or living in the dens of woodchucks.

There are no records available of fleas from *M. monax* in the most southern part of its range in the Central and Eastern States or in a great area of its range in Canada.

A new species and new genus of fleas, *Aetheopsylla septentrionalis*, was described by Stewart and Holland (1940) from a single female taken from *Marmota monax petrensis* in British Columbia. In the description the genus was not compared with *Oropsylla*. The figure and description show no characters that would distinguish the specimen from *O. arctomys*. Typical specimens of *O. arctomys* were sent to the Canadian National Museum for comparison with the holotype and no significant differences were noted. Three female specimens from the type host and type locality have been examined and are *O. arctomys*. It is the writer's opinion that *Aetheopsylla septentrionalis* is a synonym of *Oropsylla arctomys*. Mr. Holland concurs in this decision.

*M. monax*, parasitized by *O. arctomys*, is the only fossorial rodent of the family Sciuridae, a group so important in the epidemiology of sylvatic plague, that extends from the western plague infected area east to the Atlantic Coast. Plague has already been found in marmots, *M. flaviventris*, and in their parasites in western United States (Eskey and Haas, 1940). *O. silantiewi* (Wagner) and its several subspecies are the commonest flea parasites on *Marmota* in Asia (Ioff, 1925) where this mammal is an important plague reservoir. The pneumonic plague epidemics in

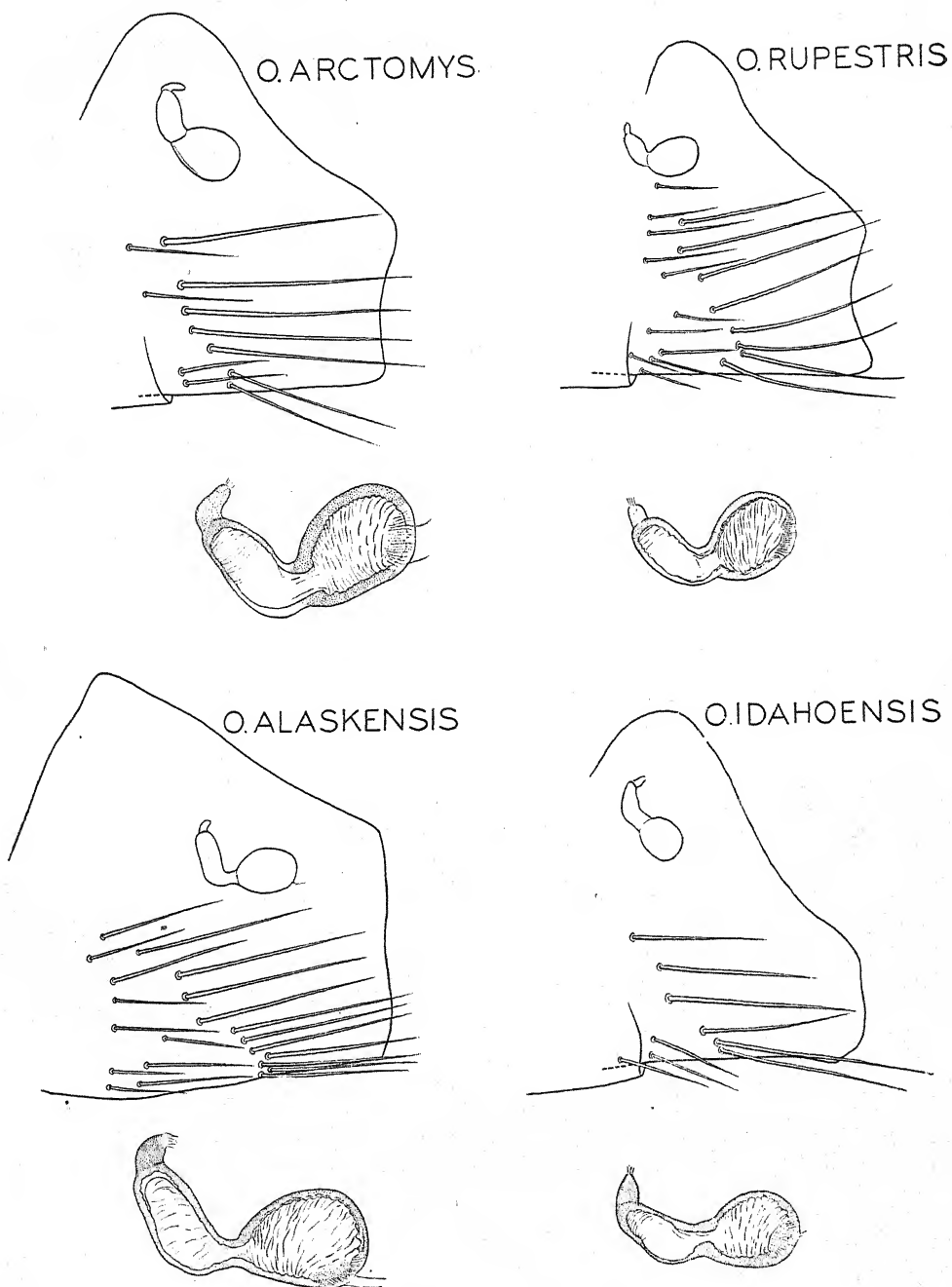


PLATE II  
Receptaculi and Sternites VII of Females

Manchuria of 1910-1911 and 1920-1921 initiated among hunters who were taking marmots or "tarbagans" for their pelts (Wu Lien-Teh et al, 1936).

*Oropsylla rupestris* (Jordan) 1929

1936. *Oropsylla rupestris* (Jordan). Ioff, Zeit. Parasit. 9: 73-124.  
 1940. *Oropsylla rupestris* (Jordan). Eskey and Haas, Pub. Health Ser. Bull. No. 254: 30, 40, 41, 58, 59, 64, 73.  
 1942. *Oropsylla rupestris* (Jordan). Jellison and Good, Nat. Inst. of Health Bull. No. 178: 120. (Bibliography up to 1939.)  
 1943. *Oropsylla rupestris* (Jordan). Ewing and Fox, U. S. Dept. of Agric. Misc. Pub. No. 500: 50.

*Male*: Length 2.1 mm, frontal tubercle small, 2 pairs preoral bristles, 1 large bristle and row of small bristles along dorsal margin of antennal groove, pronotal ctenidium of 18-20 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 1-2-2-2 respectively, basal abdominal sternite with 1 pair ventral bristles, moveable process of clasper broadly club-shaped with anterior margin convex as in *O. arctomys*. In a topotype ♂ there are two unequal acetabular bristles well separated on each side, the upper one being about  $\frac{1}{2}$  the length of the lower bristle. In all other specimens available there is only 1 acetabular bristle as figured by Jordan (1929), and as found in *O. arctomys*. Sternite VIII with 4 or 5 large sub-terminal bristles and 1 to 3 pairs of smaller ventral bristles.

*Female*: Length 3.1 mm, frontal tubercle small, pronotal ctenidium of 24 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 2-2-2-2 respectively, basal abdominal sternites with 1 lateral and a ventral pair of bristles; style  $1\frac{1}{2}$  times longer than broad, with 1 terminal and 4 lateral bristles, sternite VII with a row of 8 large and 10 or 11 scattering smaller bristles, receptaculum figured. The most reliable character for the separation of *O. rupestris* from *O. arctomys* appears to be that mentioned in the original description by Jordan, namely the relative position of the spiracle and lower bristle on tergite II, in *O. rupestris* this bristle is definitely posterior to spiracle and in *O. arctomys* it is below the spiracle. (It must be admitted that this is a very minute character.) Size and host relationship are useful in the determination of the species.

The female of *Oropsylla rupestris* (Jordan) was first described as the allotype of *Opisocrostis labis* (Jordan & Rothschild) but the error was corrected by Jordan with a description of the male and redescription of the female. Its close relationship to *O. arctomys* was noted. Specimens were recorded from *Mustela longicauda*, (= "*Putorius longicauda*"), *Citellus richardsoni* (= "*Spermophilus richardsoni*"), *Thomomys* and *Canis* in Alberta, Canada. Jordan stated that *Citellus* ("*Spermophilus*"), is probably the true host.

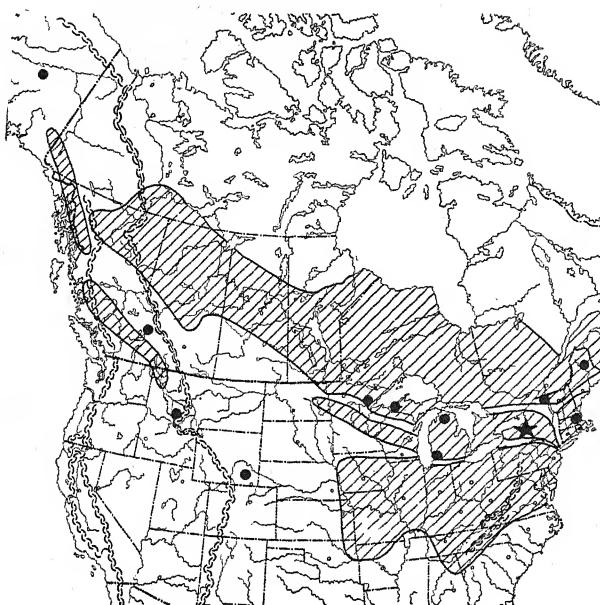
Jordan (1937) recorded 2 females of *O. rupestris* from *Marmota flaviventris* in Colorado. This locality is so far from all other collections of the species and from the range of the normal host that the record, based on females only, is questionable.

The record of *O. rupestris* from *Citellus columbianus*, Missoula County, Montana, by Jellison, Kohls and Mills (1943) based on a single ♂ specimen must also be questioned as the location is distinctly west of the range of *C. richardsoni*.

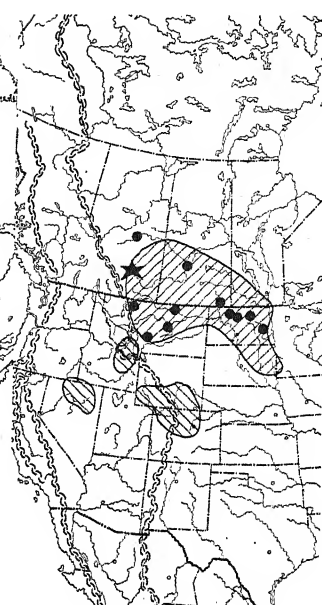
Further collections confirm Jordan's statement that this is a ground squirrel flea and furthermore its geographical distribution seems to be limited to one species of ground squirrel, i.e., *C. richardsoni*. All specimens examined by the writer from *Citellus*, where the host had been specifically determined, came from *C. richardsoni*, except the one ♂ specimen from *C. columbianus* noted above. It may also be found on *C. franklini* and *C. tridecemlineatus* where their ranges overlap the range of *C. richardsoni*, but present records indicate it has very strict host specificity.

The host relationships of this species involves a critical problem in mammal taxonomy. Howell (1938) regards the "Wyoming ground squirrel" as a subspecies of *C. richardsoni* and states that *C. r. elegans* and *C. r. richardsoni* intergrade in

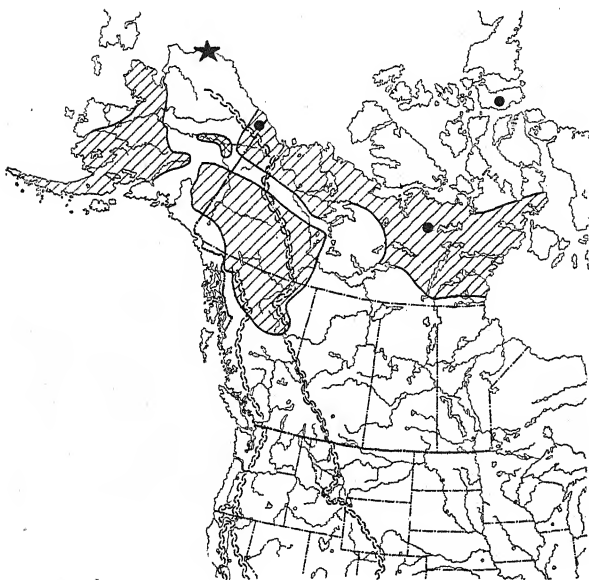
# O. ARCTOMYS



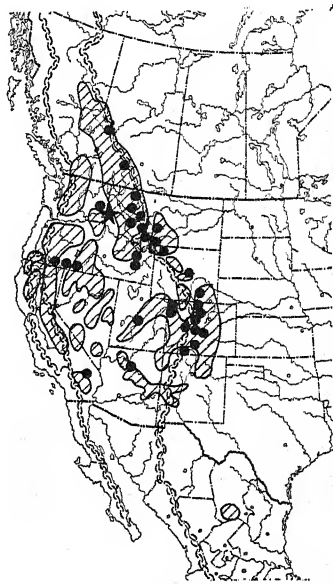
# O. RUPESTRIS



# O. ALASKENSIS



# O. IDAHOENSIS



## PLATE III

Collection records of *O. arctomys* superimposed on distribution map of *Marmota monax*, after Anthony, 1928.

Collection records of *O. rupestris* superimposed on distribution map of *Citellus richardsoni*, after Howell, 1938. Large area = *C. r. richardsoni*. Small areas = *C. r. elegans* and *C. r. nevadensis*.

Collection records of *O. alaskensis* superimposed on distribution map of *Citellus parryi*, after Howell, 1938.

Collection records of *O. idahoensis* superimposed on distribution map of *Citellus (Callospermophilus) lateralis*, after Howell, 1938.

(A star indicates type locality of the species.)

southwestern Montana, whereas Davis (1939) and Anthony (1928) consider them as distinct species. The writer has experienced much difficulty in the specific identification in the field of ground squirrels in Beaverhead, Madison, and Gallatin Counties, Montana, where these two squirrels meet and possibly intergrade. All flea specimens from typical *C. r. richardsoni* in Gallatin County have been *O. rupestris*, although the series from this county includes a few atypical specimens. A large series of specimens from what is probably *C. r. elegans* in Madison and Beaverhead counties are *O. idahoensis* as are all collections from *C. r. elegans* in Wyoming.

It is very unusual for a species of flea to be restricted to one of two subspecies of hosts which intergrade in their overlapping territory. A more exact study of this parasite-host problem would be of interest.

*O. rupestris* was found to be an efficient vector of plague under laboratory conditions and plague was reported in its natural host *C. richardsoni* by Eskey and Haas (1940).

Plague infection has been reported in ground squirrel fleas from Alberta in 1942 (Public Health Reports) within the range of *C. richardsoni* but the squirrel host was not specifically given. An endemic area of sylvatic plague covering over 2000 square miles in southern Alberta is reported by Brown and Roy (1943). This area is within the range of *C. richardsoni* and it is assumed that this is the ground squirrel reservoir concerned.

#### *Oropsylla alaskensis* (Baker) 1904

1942. *Oropsylla alaskensis* (Baker). Jellison and Good, Nat. Inst. of Health Bull. No. 178: 118. (Bibliography up to 1939.)

1943. *Oropsylla alaskensis* (Baker). Ewing and Fox, U. S. Dept. of Agric. Misc. Pub. No. 500: 49.

*Male*: Length 3.7 mm, frontal tubercle very small, preoral bristles 2 pairs, labial palpi exceeding trochanters and about  $\frac{1}{2}$  of femur, dorsal border of antennal groove with one large and many fine bristles; pronotal ctenidium of 22 to 24 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV with 1-2-2-1 respectively, 5 lateral plantar bristles on each terminal tarsal segment; basal abdominal sternite with 1 pair of ventral bristles only, antepygial bristles 1 large and 2 minute in each group. Two acetabular bristles on each side, well separated, moveable process of clasper long, anterior margin straight; sternite VIII with 4 large terminal and 2 pairs of medium ventral bristles.

*Female*: Length 4.6 mm, pronotal ctenidium of 24 to 26 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 2-2-2-0 in 1 specimen and 1-2-2-1 in another (this character is variable in many species); stylet two times longer than wide with one or two large terminal bristles and 4 lateral bristles, sternite VII not rounded at posterior dorsal corner, and with a row of 9 large and 10 small scattering bristles, receptaculum figured.

This species has been known only by the type collection which included both sexes from "*Citellus barrowensis*" = *Citellus parryi barrowensis* from Point Barrow, Alaska. This is the most northerly record of a ground squirrel flea in North America and the species is one of the largest of the ground squirrel fleas. Through the courtesy of Mr. Holland, I have seen the following specimens from the Northwest Territories, Canada:

From *Citellus*, Thelon Game Sanctuary, Northeast of Great Slave Lake, July 18, 1937, 1 ♂, 1 ♀, collected by C. H. D. Clarke.

From *Citellus*, Baker Lake, Aug. 29, 1937, 1 ♀, collected by C. H. D. Clarke.

From Arctic weasel, *Mustela arctica*, Baker Lake, Aug. 27, 1937, 1 ♀.

From Arctic fox, *Alopex lagopus inuitus*, Craig Harbour, Ellesmere Island, Nov. 17, 1934, 4 ♀.



The only species of ground squirrel in this general region, and the probable host of the flea, is *Citellus parryii parryii* according to a distribution map given by Howell (1938).

Besides the type specimens, there is also a single female of *O. alaskensis* without host data in the United States National Museum from Longitude 141°, Latitude 69°, collected by J. M. Jessup and J. M. Aldrich, August 14, 1912. This locality, near the Alaskan-Yukon border north of the Arctic Circle, is also within the range of *C. p. parryii*.

*Oropsylla idahoensis* (Baker) 1904 = *Ceratophyllus poeantis* Rothschild = *Ceratophyllus bertholfi* Fox

1936. *Oropsylla idahoensis* (Baker). Ioff, Zeit. Parasit. 9: 73-124.  
 1939. *Oropsylla idahoensis* (Baker). Jellison and Kohls, Pub. Health Rept. 54: 2022-2023.  
 1940. *Oropsylla idahoensis* (Baker). Jellison and Good, Nat. Inst. of Health Bull. No. 178: 118-120. (Bibliography and synonymy up to 1939.)  
 1940. *Oropsylla idahoensis* (Baker). Eskey and Haas, Pub. Health Bull. No. 254: 30, 32, 33, 39, 40, 41, 45, 57, 60, 64, 73, figs. 54, 55, 58, 61.  
 1940. *Oropsylla idahoensis* (Baker). Hubbard, Pac. Univ. Bull. No. 37(4).  
 1941. *Oropsylla idahoensis* (Baker). Hubbard, Pac. Univ. Bull. 37(7A): 213, and 37(8): 5.  
 1941. *Oropsylla idahoensis* (Baker). Svihla, Univ. of Wash. Pub. in Biology 12(2): 16.  
 1942. *Oropsylla idahoensis* (Baker). Augustson, Bull. South. Cal. Acad. Sci. 40: 153-154.  
 1943. *Oropsylla idahoensis* (Baker). Augustson, Bull. South. Cal. Acad. Sci. 42: 82.

*Male*: Length 2 mm, frontal tubercle small but distinct, contrary to original description, 2 pairs preoral bristles, labial palpi exceeding trochanter and about equal  $\frac{1}{2}$  of forefemur; pronotal ctenidium of 16-18 spines, apical spinelets on each side as follows: metanotum 1, abdominal tergites I to IV, 1-2-2-1 respectively, basal abdominal sternite with a pair of ventral bristles, sternite VIII small with 4 terminal bristles closely grouped and with 0, 1 or 2 pairs of smaller ventral bristles; moveable process of clasper with an obtuse angle on anterior margin not rounded as *O. arctomys* or *O. rupestris*; 2 acetabular bristles, well separated; 1 large and 1 very small antepygidial bristle, in each group.

*Female*: Length 2.7 mm, frontal tubercle small, sternite VII with a row of 6 large bristles and 3 or 4 smaller scattering bristles on each side, stylet tapering to a point  $1\frac{1}{2}$  times longer than wide, and with 1 terminal and 4 lateral bristles.

Baker described both sexes of *Oropsylla idahoensis* from specimens collected from *Citellus columbianus* at Moscow, Idaho, and it has since been recorded from many species of *Citellus* (*sensu lato*) in the western part of North America. On *Citellus columbianus* it is found in association with and usually outnumbered by *Thrassis petiolatus* (Baker), *Opisocrostis tuberculatus* (Baker), and *Neopsylla inopina* Rothschild. On most other species of ground squirrels it is likewise found in association with some species of *Thrassis*, *Opisocrostis* and *Neopsylla*. However, as suggested by Hubbard (1940), it is the only consistent parasite on the golden-mantled ground squirrel, *Citellus (Callospermophilus) lateralis*. *C. lateralis* is host to stray fleas from *Eutamias*, *Tamiasciurus* and *Citellus*, and other rodents which share the same habitat but not with the same regularity that it is host to *O. idahoensis*. The range of this flea corresponds roughly with the range of *C. lateralis* except in the extreme north where *O. idahoensis* extends into Alaska on other species of *Citellus*, while *C. lateralis* extends only into British Columbia and Alberta. It exists consistently and certainly breeds in ground squirrel populations many miles from the habitat of *Callospermophilus* yet the general distribution of the parasite and this host are certainly related. This parasite is conspicuously absent from ground squirrels in the coastal region of California where *Diamanus montanus* is so abundant, and from ground squirrels in the prairie states east of the Rocky Mountains.

The collection of specimens available contains two series of special interest, one from *Citellus* in Alaska, and the other from *Cynomys* in Colorado. The Alaskan specimens are larger, darker and have more bristles and ctenidial spines than specimens from the type locality. Sternite VIII of the male in these specimens is also wider and has some additional bristles. Those from *Cynomys*, near the southern extremity of the range of the species are also large and robust specimens. As *Cynomys* is a distinct genus, related to both *Citellus* and *Marmota* and of similar habits a distinct species of *Oropsylla* might be expected on it. The series of fleas available does not warrant a separation of another species or subspecies but emphasizes the rather wide variation and wide geographical as well as host range of *O. idahoensis*.

*O. idahoensis* is very similar to *O. iloviaskii*, a parasite of *Citellus fulvus* of the Russian Steppes, and to *O. elana* from *Citellus* in Manchuria. There is little in the published figures or descriptions to separate the three species. A series of all three forms is necessary to determine their synonymy or identity.

#### SUMMARY

The American species of *Oropsylla*, namely *O. alaskensis*, *O. arctomys*, *O. idahoensis*, and *O. rupestris*, have been reviewed. Present records indicate the following host relationships and geographical distribution:

*O. alaskensis* is restricted to the ground squirrels of the *Citellus parryii* group of the Far North, even entering the Arctic Circle.

*O. arctomys* is a characteristic parasite of the woodchuck *Marmota monax*, but only very rarely occurs on other species of *Marmota* beyond the range of *M. monax*.

*O. rupestris* appears to be limited to *C. richardsoni richardsoni*.

*O. idahoensis* shows less host specificity and is found on a great many species of *Citellus* and also on *Cynomys* in the Western States, Canada and Alaska. It is the most characteristic flea parasite of the squirrel subgenus *Callospermophilus* and its range roughly coincides with this host except in the Far North.

All species are found occasionally on rodents other than their normal hosts and frequently on predators within their range.

#### ACKNOWLEDGMENTS

The writer is indebted to the following for the loan of specimens: Mr. G. P. Holland, Livestock Insect Laboratory, Kamloops, British Columbia; United States National Museum; and Department of Entomology, Montana State College. All other specimens are from the collection of the Rocky Mountain Laboratory. Collection records tabulated or recorded on the distribution maps are from specimens reviewed by the writer in the preparation of this paper.

Drawings were made by Gretchen Gayhart Jellison.

#### REFERENCES

- ANTHONY, H. E. 1928 Field Book of North American Mammals, pp. 1-625.  
BAKER, C. F. 1904 The classification of the American Siphonaptera. Proc. U. S. Nat. Mus. 27: 121-170.  
BROWN, J. H. AND ROY, G. P. 1943 The Richardson ground squirrel, *Citellus richardsoni* Sabine, in southern Alberta: its importance and control. Sc. Agric. 24: 176-197.  
DAVIS, W. B. 1939 The recent mammals of Idaho, pp. 1-400.

- ESKEY, C. R. AND HAAS, V. H. 1940 Plague in the western part of the United States. Pub. Health Bull. No. 254: 1-83.
- EWING, H. E. AND FOX, I. 1943 The fleas of North America. U. S. Dept. Agric. Misc. Publ. No. 500: 1-142.
- HOWELL, A. H. 1938 Revision of the North American ground squirrels, with a classification of the North American Sciuridae. N. Am. Fauna, No. 56, pp. 1-256.
- HUBBARD, C. A. 1940 Fleas of the Yakima ground squirrel. Pacific Univ. Bul. 37(7A): 1-4.
- IOFF, I. 1925 Fleas of marmots (*Marmota*) and earless marmots (*Citellus fulvus*). (Translation) Revue de Mikrobiologie et d'Epidémiologie 4: 9-10.
- JELLISON, W. L., KOHLS, G. M., AND MILLS, H. B. 1943 Siphonaptera: species and host list of Montana fleas. Misc. Publ. No. 2, Montana State Board of Entomology, pp. 1-22.
- JORDAN, K. 1929 Notes on North American fleas. Novitates Zoologicae 35: 28-39.
- 1933 A survey of the classification of the American species of *Ceratophyllus* s. lat. Novitates Zoologicae 39: 70-79.
- 1937 Records and descriptions of Siphonaptera. Novitates Zoologicae 40: 283-291.
- AND ROTHSCHILD, N. C. 1911 Some new Siphonaptera from China. Proc. Zool. Soc. London, 1911, 365-392.
- STEWART, M. A. AND HOLLAND, G. P. 1940 A new genus of the Family Dolichopsyllidae (Siphonaptera) from Canada. Canadian Entom. 72: 41-42.
- WAGNER, J. 1929 Über die nordamerikanische Ceratophylli welche auf Ziesel und Murmeltieren leben. Knowia 8: 311-315.
- 1930 Katalog der Palaearktischen Aphaniptren. Wien, 1930, Verlag von Fritz Wagner.
- AND IOFF, I. 1926 Über Flöhe der Ziesel und der Springmäuse, unter Berücksichtigung der Verbreitung der Pesterkrankungen in den Wolga-Steppen. Revue de Mikrobiologie et d'Epidémiologie 5: 57-116.
- WU LIEN-TEH, CHUN, J. W. H., POLLITZER, R. AND WU, C. Y. 1936 Plague. A manual for for medical and public health workers.

# EFFECTS OF VARIOUS SULFA COMPOUNDS ON THE PROTOZOAN PARASITE, *EIMERIA TENELLA*\*

C. A. RIPSOM AND C. A. HERRICK

University of Wisconsin

## INTRODUCTION

Following the work of Horton-Smith and Taylor (1942) on the use of sulfamethazine and sulfadiazine in the control of caecal coccidiosis in chickens, the present authors endeavored to confirm and extend the information gained from their work. To do this a series of twenty-five experiments involving the use of several hundred birds was devised.

Since sulfadiazine was more readily available than was sulfamethazine at the time these experiments were conducted, emphasis was placed on the former compound.

## MATERIALS AND METHODS

The laboratory animals used were Single Comb White Leghorn chickens which were furnished by the Department of Poultry Husbandry at the University of Wisconsin. Their ages ranged from 2 days to 2½ months. The sexes of these chickens were approximately equal in number and the data were recorded separately as to sex but as no significant difference was seen, the data from both sexes in each experiment were combined in the final consideration.

The birds used were all kept in a separate house (Edgar and Herrick, 1944) and fed with sterilized feed (Fish, 1931) until needed. At this time they were removed to another building and immediately infected. Following infection they were kept under conditions as described by Herrick, Ott and Holmes (1936), i.e., sterile cages supplied with sterile feed and running water. The method of infection and the oöcysts used were as described by Edgar and Herrick (1944).

The chickens were distributed to their respective experimental cages as they were selected at random, thus making the weight and physical condition of the birds in the various cages practically identical.

The severity of the infection and the therapeutic value of the treatment of the birds was evaluated in four ways: (1) daily observations of clinical symptoms, (2) weight changes from the beginning to the end of the experiments, (3) feed consumption, and (4) the number and size of the caecal lesions at autopsy (Herrick, Holmes and DeGiusti, 1942).

Administration of sulfadiazine was by: (1) 0.5-gram pills, (2) aqueous suspension of the powdered form, (3) powder in appropriate sized gelatin capsules, and (4) mixed in the feed. The sulfadiazine suspension was prepared by mixing 100 grams of the powder with 250 cc. of distilled water. It was then administered into the crop by means of a calibrated syringe, care being taken that each bird received its proper dose. The amount of the compound given was dependent upon the weight of the bird since they were treated at the rate of 0.0128 grams of sulfadiazine per gram weight of bird.

Received for publication, December 5, 1944.

\* Published with the permission of the Director of the Agricultural Experiment Station. This work was supported by the Dr. L. D. LeGear Medicine Company and also by the Wisconsin Alumni Research Foundation. The sulfadiazine was furnished by the Lederle Laboratories.

To determine the number of oöcysts which were produced and sporulated after treatment of the chicken with sulfadiazine, the oöcysts were concentrated by means of the modified Lane technique (Lane, C., 1923-1925). Fifty high-power (4-mm) objective fields of the supernatant in the tubes used in the concentration process were examined by placing a drop of the material on a regular glass microscope slide. Counts were then made of the number of sporulated and unsporulated oöcysts, gametocytes and imperfect forms; the results were recorded separately. From these figures the percentage of sporulation of the oöcysts was found. In those cultures showing no oöcysts on first examination, two more slides of the same supernatant fluid were examined to insure that no forms had been missed.

#### PRESENTATION OF DATA

The data on administration of sulfadiazine will be presented under two main headings: (1) single dosage and (2) in the feed. Prevention, cure and sporulation effect will be considered in that order. Observations on other data and on the use of other compounds will be noted as addenda.

In preliminary work to determine the minimum lethal dose of sulfadiazine for chickens, it was found that they had considerable tolerance for the compound. As much as 12 grams were given to birds weighing from 400 to 600 grams without causing death. The salient feature of this test was in finding that sulfadiazine, when given in large doses, resulted in a decided and rather rapid weight loss. The crop contents became more solidified than in normal birds, and the animal apparently starved due to an inability to carry through the normal digestive processes.

In subsequent experiments it was found, concomitantly with the aforementioned starvation, that the caecal pouch contents were quite abnormal both in consistency and in color. Normally the caecal pouch content is a finely granular substance containing many fat droplets when seen under the microscope and teems with bacteria of a wide variety. After treatment with sulfadiazine the pouch contents were moderately coarse and flaky and of a darker brown color than normal. The bacterial flora was greatly reduced in total number and the most conspicuous organism was a short, thick, motile bacillus. No attempt was made in these experiments to identify the bacteria found.

To determine the optimum dose to be used in subsequent trials, the sulfadiazine was administered in varying amounts to seven groups of birds, the lowest level being 0.0004 gm of sulfadiazine per gram weight of bird and this dosage was increased in multiples of two to each succeeding group. The maximum level used was 0.0256 gm; the optimum was found to be 0.0128 gm.

#### PREVENTION BY SINGLE DOSE

Having found a wide margin of safety in treatment, an experiment was devised to determine how long a single dose of 0.0128 grams of sulfadiazine per gram weight of bird would be maintained at a protective level in birds of from 400 to 600 grams weight and what effect such treatment might have on their weight changes.

The birds used in this experiment were divided into nine groups, two of which were used as (1) infected, untreated controls and (2) uninfected, untreated (fully normal) controls. The other seven groups were given the sulfadiazine for varying lengths of time before infection. One group was treated 6 days prior to infection, one 5 days before infection and so on until that group was reached which was given



the sulfadiazine and the infection at the same time, the "zero-day" group. At the time the zero-day group was infected, all other groups were also infected except the fully normal controls. Each bird was given 400,000 oöcysts. Seven days later the birds were all killed to determine those which had resisted infection.

As seen in Table 1, none of those birds treated on zero-day took the infection. Of those treated 1 full day before infection, one-half were negative and one-half were severely infected. In the group treated 2 full days prior to infection, 1 bird was negative, 1 had a 2-plus infection and 2 had a 4-plus infection. All the chickens treated 3 or more days before being given oöcysts developed severe caecal lesions as did the infected, untreated controls. The fully normal birds were all negative. It seems, therefore, that the sulfadiazine in large doses remained at a fully protective

TABLE 1.—Illustrating the length of time a single dose of 0.0128 grams of sulfadiazine per gram weight of bird remains effective as a preventive against 400,000 oöcysts of *E. tenella* and how it affects the weight gains of the treated chickens

Use of bird	Group No.	Severity of infection	No. of birds used	Average initial weight	Average terminal weight	Average daily gain	No. of days treated with sulfadiazine before autopsy*
				gm	gm	gm	
Uninfected untreated (fully normal) control	1	—, —, —	3	596.6	833.6	17.9	0
Infected untreated control	2	4+, 4+, 4+	3	586.0	629.6	3.3	0
"0" days	3	—, —, —, —	4	553.5	653.0	14.6	7
1 day	4	—, —, 4+, 4+	4	567.0	655.0	10.9	8
2 days	5	—, 2+, 4+, 4+	4	565.7	661.7	10.6	9
3 days	6	+, 3+, 4+, 4+	4	455.7	502.7	4.7	10
4 days	7	2+, 4+, 4+, 4+	4	506.0	643.0	7.0	11
5 days	8	4+, 4+, 4+	3	545.0	594.0	4.1	12
6 days	9	3+, 4+, 4+	3	508.3	522.6	1.1	13

\* Autopsied 7 days following infection.

level for a comparatively short period of time. Treatment and infection at the same time, zero-day, was the only time of treatment which could be depended upon to prevent the infection. In the birds treated 1 day before infection the protection was not constant. In those treated 2 days before infection the protection was doubtful, and in those treated 3 or more days before infection, no protection could be demonstrated. Protection by sulfadiazine is probably effected by an attack on the sporozoites of *E. tenella* when they are released from the oöcysts.

Comparing the weight changes of the different groups (Table 1), one can see a general decline in the average daily gains from those birds having had sulfadiazine for only 7 days, the zero-day group, to those having had it for 13 days, the 6-day group. It seems that the difference in weight gains can be attributed to the effect of the sulfadiazine. It was most noticeably seen between the zero-day group, the infected controls, and the 6-day group. The weight gain difference between the infected and the uninfected controls is obviously due to the infection. The protocol of this particular experiment precluded the possibility of following the weight changes for a longer time period. It was believed, however, that the failure to gain weight continued with extended time until the weight changes were in the negative values. Rough experiments which followed substantiated this view. Birds tested on this

point did lose weight for a variable length of time but given sufficient time (several weeks) were able to overcome entirely the effect on their weight and rival the weight of the fully normal control birds.

#### THERAPEUSIS BY SINGLE ADMINISTRATION

Having determined the protective capacity of sulfadiazine against infection with *E. tenella*, an attempt was next made to establish its therapeutic value. To accomplish this end an experiment was set up on much the same plan as that used to demonstrate protection. A number of 2-day-old birds were divided into nine groups one of which acted as the fully normal controls. The remaining eight groups were infected at zero days with 50,000 sporulated oöcysts. Treatment was with a single 0.5-gram tablet of sulfadiazine and was instituted at the time of infection, zero-days, for group 1, 9 hours following for the second group and so on until the number 5 group was treated with the sulfadiazine 3 days after the oöcysts had been adminis-

TABLE 2.—Illustration of the comparative efficacy of sulfadiazine in single doses when given at various times following infection in curing coccidiosis in chickens 7 days old

Group number	Time of treatment in relation to time of infection <sup>††</sup>	Number of birds used	Severity of lesions					
			—	1+	2+	3+	4+	Others
1	At the same time <sup>†</sup>	10		2	4	3	0	1*
2	9 hrs. post-infection <sup>†</sup>	10	2	1	1	0	6	0
3	1 day following <sup>†</sup>	10	1	1	2	4	0	2†
4	2 days following <sup>†</sup>	10	1	3	4	1	0	1*
5	3 days following <sup>†</sup>	10	8	1	0	1	0	0
6	4 days following <sup>†</sup>	10	3	4	3	0	0	0
7	5 days following <sup>†</sup>	8	1	1	2	4	0	0
8	0.5 gm pill daily for 5 days	10	6	4	0	0	0	0
9	Infected untreated controls	10	0	5	2	3	0	0
10	Fully normal controls (uninfected untreated)	8	8	0	0	0	0	0

† Dead from causes other than coccidiosis.

\* Lost wing bands.

† Given a single dose of 0.5 gm of sulfadiazine.

†† Given 50,000 oöcysts.

tered. Group 8 was treated with 0.5 gram of sulfadiazine at the time of infection and each day thereafter for 5 days. Two days following the last treatment with the compound (7 days following infection), all the birds were autopsied in order to learn the therapeutic effect of the sulfadiazine as indicated by the severity of the caecal lesions.

By consulting Table 2, it can readily be seen that those birds treated on the third day following infection were the ones afforded the greatest protection by a single dose. Treated statistically, these data indicate that a cure of over 80% was effected by treatment on this day. It is questionable whether those birds treated at any later time were benefited by the treatment. In later experiments in which 1% sulfadiazine was mixed in the feed, each bird actually got about twice as much sulfadiazine per day as those getting a single 0.5-gram tablet per day. The failure in the zero-day group (group 1, Table 2) to resist infection must be ascribed therefore to the low level at which the sulfadiazine was given. As shown in Table 2, group 5 received 0.5 gram of sulfadiazine on the third day following infection and the life cycle of the parasite was disrupted in the first generation merozoite stage. Likewise group 8, which received 0.5 gram daily for 5 days, were benefited by inhibiting the same stage.

However, when this amount of sulfadiazine was given either at the time of infection or 9 hours following infection, when the sporozoite stage was present, little benefit was afforded. Since the sporozoites and the merozoites were both subjected to the same level of the drug and the sporozoites persisted while most of the merozoites were killed, it indicates that the sporozoites have a higher threshold of tolerance than do the merozoites. This is, however, not so surprising since in other sporozoan infections (malaria), particular stages of the parasite are more readily attacked by certain chemicals than are other stages.

#### SPORULATION

During routine autopsy, the caecal pouch tissues of several birds that had been treated with sulfadiazine during the sixth day of infection were examined and the caecal pouch contents were set away to sporulate (see Materials and Methods) for 24 hours. Contrary to expectation, no gametocytes or unsporulated oöcysts could

TABLE 3.—*Demonstrating the effects of a single large dose of sulfadiazine on production of and sporulation of oöcysts when given after the symptoms of coccidiosis appeared*

No. of birds used	Use of birds	No. of oöcysts counted	No. of oöcysts sporulated	No. of gametocytes or other forms counted	Percentage of sporulation	No. of high-power (4-mm obj.) fields counted	Average dose of sulfadiazine given
9 a*	Test	0	0	29	0.0	25	gm 9.9
1 a	Infected Control	360	268	92	80.0	14	0.0
8 b†	Test	0	0	10	0.0	50+	10.4
2 b	Infected Control	369	286	83	77.5	25	0.0
8 c††	Test	97	52	45	53.0	25	8.8
2 c	Infected Control	154	125	29	80.0	25	0.0
3	Fully normal Control	0	0	0	0.0	25	0.0

\* Treated with sulfadiazine 6 days following infection.

† Treated with sulfadiazine 6 & 7 days following infection.

†† Treated with sulfadiazine 7 days following infection.

be found either in the scrapings of caecal epithelium or in the caecal contents. After allowing the caecal pouch contents to sporulate for 24 hours, a rigid examination was made of the culture after concentrating any oöcysts present by the modified Lane technique (l.c.). No form of the parasite could be found in the test birds, whereas in the normally infected control group not having had treatment, there were between 10 million and 15 million oöcysts per cc of the material brought to the surface. The counts were made by dilution using the Spencer Bright-Line Haemocytometer. Examination of the birds treated with sulfadiazine at the end of the sixth day showed normal numbers of oöcysts with a fairly high percentage of sporulation. The same was true of birds treated with sulfadiazine during the fifth day of infection. These data, considered in the light of work done by Edgar, Herrick and Fraser, 1944, on the development of *E. tenella*, pointed to the possibility of the sulfadiazine attacking the parasite when it is free as the second generation merozoite in the lumen of the caecal pouch. With this lead, more trials were conducted to test the efficacy of sulfadiazine in preventing the formation of oöcysts after the infection had become firmly established.

Uninfected birds 88 days old were infected with 50,000 sporulated oöcysts and then divided into three groups. Controlling these groups were an infected untreated

control group and a group of fully normal birds, i.e., untreated and uninfected. All groups to be given treatment were dosed with 0.0128 gram of sulfadiazine per gram weight of bird. Group 1 was treated on the sixth day following infection, the second group was given its treatment on the sixth and seventh days, and the third group was treated on the seventh day of infection only. The compound was given as a single dose except in group 2 where one-half of the full treatment was given each of 2 successive days.

The infection was established and the second generation merozoites escaped from the host cells as was evidenced by the copious hemorrhage on the fifth day. By the seventh day of infection these symptoms had disappeared and all of the birds recovered. Ten days from the time of infection all of the birds were killed for examination of caecal lesions and sporulation of any oöcysts that might be present in the caecal pouch contents.

As shown in Table 3, those birds given sulfadiazine at the beginning of the sixth day and also the group treated on the sixth and seventh days produced a small number of oöcysts and gametocytes, neither of which were capable of sporulation. Those birds treated on the seventh day only and the infected, untreated controls, produced a fair number of oöcysts which sporulated. The fully normal controls showed no forms whatsoever, of *E. tenella*.

From this it can be concluded that the sulfadiazine exerts its influence on the second generation merozoites, and possibly on the developing gametocytes as well. Cytological inquiry is in progress on this latter point. It can be said that treatment with sulfadiazine as late as 5 days and 22 hours following infection, when the bird was suffering severe caecal hemorrhages, resulted in failure of the parasite, *E. tenella*, to complete its life cycle. In the few cases where oöcysts were produced, they did not sporulate.

#### SULFADIAZINE IN THE FEED

Finding individual treatment with sulfadiazine too expensive of time, it was decided to try using various percentages of the compound in the feed and still accomplish the same ends as those produced by single doses. The purpose was to determine: (1) the level at which it could be given without causing excessive deleterious effects, (2) its protective capacity, (3) its therapeutic value, and (4) its effect on production and sporulation of oöcysts. To determine these points, several experiments were set up.

To learn the best protective level of sulfadiazine, 82 birds averaging 198 grams each were given various percentages of the compound in their feed. There were 7 groups in all, the infected untreated controls, the uninfected untreated (fully normal) controls, those having 1%, 0.5%, 0.25%, 0.125% and 0.062% of sulfadiazine in the feed in the respective groups. Twenty-four uninfected birds were distributed equally among the cages getting the respective levels of sulfadiazine so as to have uninfected treated controls for each level. All save the uninfected control birds were given 300,000 sporulated oöcysts immediately after starting them on the treatment. Sulfadiazine was continued in the feed for the duration of the experiments, i.e., 7 days following infection, at which time the birds were autopsied. The results of this experiment, as shown in Table 4, indicate that group 1 (1% sulfadiazine) was fully protected, group 2 (0.5% sulfadiazine) was one-half protected and one-half took a light infection. In group 3 (0.25% sulfadiazine) 6 had severe caecal lesions, 1 had moderate lesions and 1 was negative. Levels of the compound of 0.125% or below

allowed moderate to severe infections in all cases. None of the uninfected controls became accidentally infected. This demonstrated the unreliability of levels below 1% in ability to protect and that 1% afforded complete protection from infection with *E. tenella* when treatment began before or at the time of infection.

Next an experiment was set up to learn if levels of sulfadiazine higher than 1% would be injurious. In this test, birds 42 days old and averaging 206.3 grams each were divided at random into 8 groups (see Table 2). Infection was with 160,000 sporulated oöcysts, the infectivity of which had been previously tested.

TABLE 4.—*Illustrating the protection afforded chickens against infection by E. tenella with varying amounts of sulfadiazine*

Per cent of sulfadiazine used	No. of birds used	Birds used as	Time (in hours) when sulfadiazine was given			Pathology of caecal lesions
			Before infection	After infection	Total time (hr.) of being fed sulfadiazine	
1.0	10	Test	0	0	168	10 neg.
	8	Test				8 neg.
	4	Treated uninfected	24	168	192	4 neg.
0.5	7	Test				—, —, —, —, —, —
	4	Treated uninfected	24	168	192	4 neg.
0.25	8	Test				4+, 4+, 4+, 4+, 4+, 4+, 2+, —
	4	Treated uninfected	24	168	192	4 neg.
0.125	8	Test				2+, 2+, 4+, 4+, 4+, 4+, 4+
	4	Treated uninfected	24	168	192	4 neg.
0.062	8	Test				2+, 2+, 4+, 4+, 4+, 4+, 4+
	4	Treated uninfected	24	168	192	4 neg.
0.0	8	Infected but not treated				All (8) +
	4	Uninfected untreated (fully normal)	0	0	0	All negative

Treatment with sulfadiazine was started in the 3 test groups and their infected cage mate controls, at 1%, 3%, and 5% levels, respectively. This treatment began 5 days after giving the oöcysts and was continued for 5 days thereafter when the birds were autopsied. The severity of caecal infection was then noted and the caecal pouch contents were put aside for 24 hours to allow any oöcysts present to sporulate. Weights of each bird were taken 1 day before infection, at the time of treatment, and on the tenth day (at autopsy).

The data from this experiment are consolidated in Table 5 where the gross pathology, the weight changes and the effect on the production and sporulation of



TABLE 5.—Illustrating the effect of sulfadiazine on *E. tenella* when incorporated in the feed during the fifth and sixth days following infection

Use of bird	No. of birds	Group No.	Severity of infection	Daily av. wt. chg. dur. 1st 5 days	Daily av. wt. chg. 2nd 5 days	Daily av. wt. chg. 10 days	Percentage of sporulation of oöcysts	No. of oöcysts	Deaths between 5 & 10 day infect.
Fully normal control	8	1	All neg.	gm. 3.6	gm. 5.55	gm. 4.6	0	0	0
Infect. un- treat. controls	4	2	4+, 4+, 4+, 1+	5.8	-3.5	1.15	75%	Many	0
1% feed uninfected	3	3	All neg.	7.4	5.5	5.5	0	0	0
1% feed infected	7	4	4+, 3+, 3+, 3+, 4+, 4+	4.6	2.4	3.14	0	0	(28.5%) 2
3% feed uninfected	4	5	All neg.	8.3	-3.55	2.1	0	0	0
3% feed infected	6	6	4+, 3+, 4+, 4+, 4+, 4+	8.3	-7.0	1.3	0	0	(50%) 3
5% feed uninfected	3	7	All neg.	6.0	-5.7	+0.23	0	0	0
5% feed infected	7	8	4+, 4+, 4+, 4+, 4+, 4+	2.54	-4.4	-0.85	0	0	(71%) 5

oöcysts are noted. All birds given the infection showed typical symptoms whereas those not given oöcysts were negative. The sulfadiazine in none of the three percentages used can be said to have a measurable value in alleviating the symptoms of the developing disease but no oöcysts could be found in the caecal pouch contents of any of the treated birds. In the untreated birds, on the contrary, a large number of oöcysts were present in the caeca and these oöcysts sporulated normally. Although the sulfadiazine prevented the production of oöcysts, it can be seen (Table 5) that treatment with the higher percentages (3% and 5%) resulted in more severe weight losses than were caused by the infection alone. This is in accord with the work of Schmidt et al, 1944. It can be concluded, then, that the higher levels did adversely affect the weight gains of the birds and that none of the levels had any visible effect on the clinical symptoms of the disease but all concentrations did prevent the production of oöcysts. It is conceivable that if the sulfadiazine is given even after the appearance of blood in the faeces, it may be of considerable value in preventing an epidemic. This would be accomplished by preventing infection in those not infected and by preventing those already infected from becoming carriers.

After it was discovered that sulfadiazine prevented the development of oöcysts, the question arose as to the ability of the compound to prevent the sporulation of oöcysts after they had already become formed in the caecal epithelium. To answer this question, a group of birds was given a sub-lethal dose of oöcysts and the infection was allowed to proceed for 13 days. At this time all the birds were passing a considerable number of oöcysts. The group was then divided in half, one-half being given 1% sulfadiazine in its feed, and the other half continued on normal sterile feed. After feeding the one group sulfadiazine for 3 days, all the birds were killed and their caecal pouch contents sporulated. The ensuing oöcyst count revealed no significant difference between the test birds and their controls either in the total number of oöcysts produced or in the percentage of their sporulation. From this and other related tests it was assumed that once the oöcyst had formed, it was no longer subject to the action of sulfadiazine.

#### IMMUNITY

In order to determine the rôle of oöcyst formation in development of resistance to subsequent infection, the following experiment was set up:

The birds used were divided at random into 3 groups: (1) the fully normal controls, (2) the infected untreated controls, and (3) the infected test birds. The test birds and the infected controls were each given 50,000 oöcysts. Treatment with 1% sulfadiazine was initiated in the test birds at the beginning of the sixth day and was continued for 24 hours thereafter at which time the feed containing sulfadiazine was replaced by normal sterile feed. The infected untreated controls and the fully normal controls were kept together after the fifth day of infection.

Fifteen days following the first infection with 50,000 oöcysts, a second infection of 200,000 oöcysts was given to all the birds. Six days following the second infection, all the birds were killed in order to examine the caecal lesions which were used as an index of the presence or absence of an immunity to *E. tenella*.

The resulting data (Table 6) showed the severity of infection from the second dose of oöcysts to be almost the reverse in the 3 groups as that which was produced by the first infection. The former fully normal controls all had very severe infections, the former infected controls ranged from a heavy infection to negative and the

TABLE 6.—Illustrating that gametogeny was not an important factor in the development of resistance to subsequent infections with *E. tenella*

Group	No. of chicks	Treatment at time of 1st infection with 50,000 oöcysts	Symptoms or lesions from 1st infection	No. of oöcysts given at second infection	Caecal lesions from second infection	Average daily wt. gains in gm over 21 day period	Days from beginning of experiment to autopsy
1	5	Uninfected untreated	Symptoms	200,000	1-3+ 4-4+	6.7	21
			None				
1A	6*		Lesions				
			None				
2	6	Infected untreated	Symptoms	200,000	1-neg. 4-1+ 1-3+	8.0	21
			Medium to severe				
2A	6*		Lesions				
			1-2+ 1-3+ 4-4+				
3	6	Infected treated	Symptoms	200,000	2-neg. 1-1+ 3-2+	9.0	21
			Medium to severe				
3A	6*		Lesions				
			1-2+ 5-4+				

\* These birds were autopsied on the 6th day of infection to indicate the probable severity of the initial infection in those chickens given a second infection.

sulfadiazine test birds ranged from a moderate to a negative infection. From these data it was concluded that the development of resistance to infection with *E. tenella* was not a function of the gametogenous phase in the life cycle of *E. tenella*.

#### OTHER COMPOUNDS TESTED

Believing sulfadiazine to be peculiar in its ability to entirely protect a bird from infection, to arrest the development of the infection on the third day and again at the beginning of the sixth day, several other compounds were tested in a like manner for the same properties. These compounds were sulfathiazole, sulfaguanidine, sulfonilamide, succinyl-sulfonilamide, lime sulphur, tetraethylthiuram monosulphide and barium antimonyl tartrate. Of these, sulfathiazole showed the most promise of comparing favorably to sulfadiazine when treatment was started at the time of infection. Accordingly, it was given further tests at 1% and 2% levels in the feed. It proved to be able to prevent the infection at either level when given from the time of infection onward for 10 days, the duration of the experiment. When given after the infection had once started, however, it failed to stop the course of the disease.

The other sulfa compounds tested showed little promise of having any closely comparable properties to those found in sulfadiazine. Because of this, further experiments were not carried out with them.

The barium antimonyl tartrate was suggested by a worker in the field since some of the tartrates are used in other protozoan infections as well as in some helminth infections in fowls. It was given as single doses and at various percentages in the feed but did not prevent infection in either case. In all the tests it proved far too toxic for further consideration.

Herrick, Holmes and DeGiusti (1942) found tetraethylthiuram monosulphide to have protective properties against *E. tenella* infection in the chicken with a 20:1 margin of safety in dosage. The experiments in this series, however, showed the compound to have a much smaller margin of safety. It was difficult to give a dose small enough in young chicks to avoid killing them within a day or two. This can probably be explained on the basis of the increased age of the compound. Aging seems to decrease its protective power and increase its toxicity.

## SUMMARY

1. Sulfadiazine when given at the time of infection was an effective preventive when given as 1% of the feed or in single large doses.
2. When given during the third day of infection it materially reduced the severity of infection.
3. Given during the fifth and six days of infection it prevented oöcyst production.
4. At 2% or 3% levels in the feed it had a marked deleterious effect on the experimental animals.
5. It remained at a protective level in the chicken for not more than 1 day when given as a single large dose.
6. It did not influence the viability of the oöcysts after they had once formed in the tissues of the host.
7. Preventing the development of oöcysts did not influence the development of immunity.
8. Sulfathiazole prevented infections when given before or at the time of infection but had no effect after the sporozoites had become established.

## REFERENCES

- ALLEN, REX W. AND FARR, MARION M. 1943 Sulfaguanidine as a prophylactic during the period of acquirement of resistance by chickens to cecal coccidiosis. *Am. J. Vet. Res.* 4: 50-53.
- EDGAR, S. A. AND HERRICK, C. A. 1944 Feeding habits in relation to the severity of cecal coccidiosis, *Eimeria tenella*. *Poultry Sci.* 23: 1.
- , AND FRASER, L. A. 1944 Glycogen in the life cycle of the coccidium, *Eimeria tenella*. *Tr. Am. Micr. Soc.* 63: 199-202.
- FISH, F. 1931 The effects of physical and chemical agents on the oöcysts of *Eimeria tenella*. *Sci.* 73: 292-293.
- HERRICK, C. A., HOLMES, C. E. AND DEGIUSTI, D. L. 1942 Experimental use of organic sulphur compounds for prevention of caecal coccidiosis in chickens. *Am. J. Vet. Res.* 3: 117-127.
- , OTT, G. L. AND HOLMES, C. E. 1936 The chicken as the carrier of the oöcysts of the coccidia, *Eimeria tenella*. *Poultry Sci.* 15: 322-325.
- HORTON-SMITH, C. AND TAYLOR, E. L. 1942 Sulphamethazine and sulphadiazine treatment in caecal coccidiosis of chickens—a preliminary note. *Vet. Record* 54: 516.
- 1943 Saturated solution of sulphamethazine as a substitute for drinking water in the treatment of caecal coccidiosis in chickens. *Vet. Record* 55: 108-110.
- LANE, C. The mass diagnosis of ankylostoma infestation, 1-14. *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1923-1925, vol. 16-19.
- MAYHEW, ROY L. 1934 The present status of our knowledge of coccidiosis in chickens. *Poultry Sci.* 13: 296-297.
- SCHMIDT, L. H., HUGHES, HETTIE B., BADGER, ELIZABETH A. AND SCHMIDT, IDA G. 1944 The toxicity of sulphamerazine and sulphamethazine. *J. Pharm. and Exper. Therap.* 81: 17-42.
- , SESLER, CLARA L. AND HUGHES, HETTIE B. 1944 The chemotherapeutic activities of sulphamerazine and sulphamethazine. *J. Pharm. and Exper. Therap.* 81: 43-56.

## THE LARVAE OF *EUSTRONGYLIDES IGNOTUS* IN *FUNDULUS HETEROCLITUS*

R. P. CULLINAN<sup>1</sup>

Dept. of Biology, The Catholic University of America, Washington, D. C.

*Eustrongylides* larvae occur in *Fundulus diaphanus* (Chapin, 1926; Mueller, 1934), in *Fundulus heteroclitus* (von Brand, 1938), as well as in a variety of other fish (Hunter, 1937, 1942; von Brand, 1944). It is not possible to decide at the present time whether they all belong to one species, since the larvae of the various species are extremely similar. Von Brand and Simpson (1944), however, succeeded in bringing one specimen isolated from *Fundulus heteroclitus* to molt *in vitro*, and this worm was definitely identified as *Eustrongylides ignotus*. *Fundulus* is heavily infected with this parasite in the vicinity of Baltimore and it appeared worthwhile to study the occurrence of the worm in this fish on a large amount of material.

### MATERIAL AND METHODS

The fish were procured between September 1941 and March 1942 from two bait fishers who caught *Fundulus* in large numbers from Chesapeake Bay in the immediate vicinity of Baltimore, Md. These dealers had usually, with the exception of January and February, large stocks of freshly caught fish at hand from which representative samples were purchased and brought to the laboratory where they were kept in aquaria until dissected. Due to the fact that *Fundulus heteroclitus* was most easily obtained, the investigation was restricted to this species. It is admitted, however, that some *Fundulus macrolepidotus* may have slipped in. These two species are extremely difficult to distinguish, but it is not believed that serious errors resulted from this possibility, since *Fundulus macrolepidotus* is often not considered as a distinct species, but only a variety of *Fundulus heteroclitus*.

All the dissected fish were classified as to sex and size, the latter by measuring them from the tip of the snout to the end of the caudal fin. The sex was determined by inspection of the gonads; in very small fish the gonads look macroscopically very much alike, but it was always possible to come to a decision with the help of a microscope. In so far as the parasites were concerned, notation was taken from their number in infected fish, their location in various organs and of their length. Because of the elasticity of the worms these measurements were somewhat difficult. The centimeter rule was always kept moist in order to insure against the worm adhering to it when extended. After being stretched on the rule by means of two forceps, it was allowed to contract until it just began to deviate from a straight line. This was taken as its normal length. All measurements, both of the host and parasite, were made to the nearest tenth of a centimeter.

### RESULTS

A total of 3,507 fish was examined. Of these 465 (13.3%) were infected. Out of the total, 1589 (45.3%) were males, 1918 (54.7%) females. Parasites were

Received for publication, February 8, 1945.

<sup>1</sup> This paper, prepared under the direction of Dr. Theodor von Brand, is based on the author's dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science.



found in 208 males (13.1% of the total males, 44.7% of the parasitized fish) and in 257 females (13.4% of the total females, 55.3% of the parasitized fish). There exists, therefore, no sex difference in the degree of parasitization.

In order to study whether a relation exists between the size of the fish and the size of the parasites, the fish were divided into size classes with the difference of 1 cm between the classes. Table 1 shows the relationships between the average size of the parasites and the size of the fish on the one hand, and the percentage of infected fish in each class on the other. It is obvious that smaller fish have smaller parasites. The average size of the worms remained unchanged only when the fish reached a length of about 9 cm. The growth rate of *Fundulus heteroclitus* has not yet been studied; it is, therefore, not possible to correlate these findings with the age of the fish. Dr. S. Hildebrand (personal communication) considers a *Fundulus* of about 5 cm as about 1 year old, although considerable variations in growth due to environmental conditions seem to occur. But it appears on the whole obvious that younger fish have on the average smaller parasites. This seems to indicate rather definitely that the worms grow over a rather long period, perhaps a year or two.

TABLE 1.—Relation between size of *Fundulus*, degree of parasitization and size of worms

Length of fish, cm	Average length of worms, cm	No. of positive fish	No. of negative fish	Percentage of parasitism
3-4	4.5	11	247	4
4-5	6.9	72	730	9
5-6	7.0	98	815	11
6-7	8.1	74	617	12
7-8	8.5	105	524	17
8-9	9.4	70	142	33
9-10	10.5	18	14	56
10-11	9.2	3	3	50
11-12	10.6	2	0	100

But even if the age of the fish were exactly known, it would not be possible to draw very definite conclusions about the growth rate of the parasite from field observations alone. The last column of Table 1, showing the percentage of infected fish indicates that the larger the fish become, the greater is the degree of parasitization. This remains true, even if we disregard the two largest size classes for which only a negligible number of fish was available. The entire situation indicates that fish of various size may contract infections and that, apparently, no age immunity develops.

Another possible interpretation for the observation that smaller fish harbor on an average small parasites, could be found in the assumption that the former do not afford sufficient space or food to allow the same growth of the parasites as larger fish. This, however, seems not likely, because some small fish have very long parasites; for example a worm 10.5 cm long was found in a fish of 4.2 cm; in another instance a 14-cm worm occurred in a 5-cm fish. It seems most likely that these fish acquired their infection at an extremely young age.

The question whether the incidence or the size of the parasites varies at different seasons cannot be decided with the material available; a study extending over several years would be required.

Of some interest are the observations made on the occurrence of multiple infections (Table 2). By far the greatest majority of the infected fish harbored only one worm, but two or three parasites in one host were not too rare. The maximum

TABLE 2.—Number of single and multiple infections

No. of worms per fish	No. of fish	Percentage of total infected fish
1	352	75.7
2	60	12.9
3	30	6.5
4	11	2.4
5	4	0.9
6	1	0.2
7	2	0.4
10	3	0.6
13	1	0.2
25	1	0.2

was 25 worms in a single fish. Multiple infections were encountered in every size group of fish, but the available material is not large enough to decide whether their frequency varies with the size of the host. The length of the parasites, in cases with two or three worms in one fish, was usually fairly close. A few examples may illustrate this: two worms recovered from one fish measured 10.2 and 14.0 cm; or in another case 1.3 and 2.7 cm. Exceptions, however, occur. Thus, the length of three parasites found in one host varied in one case between 1.3 and 7.0 cm, or in another case between 2.0 and 9.2 cm. In the heavier multiple infections the size range of the worms was usually wide. In the fish having 25 parasites, the smallest one measured 2.9, the largest one 13.5 cm. The most probable explanation for these observations is that the infections were contracted at the same time in those cases where the parasites had about the same length, while superinfection must be assumed when large differences in size did occur. If this explanation is correct, it would appear that the *Eustrongylides* infection in *Fundulus* does not give rise to immunity.

TABLE 3.—Distribution of worm cysts within the fish body

Site of cyst	Number of cysts	Percentage of total cysts
Mesentery .....	602	90.4
Liver .....	40	6.0
Fat tissue .....	12	1.8
Peritoneum .....	9	1.4
Capsule of ovary .....	3	0.5

A final word may be said about the localization of the parasites within the body of the host. They were found invariably encapsulated in cysts. Most of the cysts occurred along the mesenteries (Table 3). Not a single instance was encountered in which the parasites had settled down within the muscles. This is noteworthy since in other fish species *Eustrongylides* larvae do occur rather regularly in this site (Hunter, 1942; von Brand, 1944).

## SUMMARY

1. Both sexes of *Fundulus heteroclitus* are equally parasitized by larval *Eustrongylides ignotus*.
2. The relationships between the size of the fish and the worms seem to indicate a rather slow growth of the latter.
3. Multiple infections are not very rare. Some of them are apparently acquired at the same time, while others indicate the occurrence of superinfection.
4. By far the largest number of worms is found in cysts along the mesenteries.

## BIBLIOGRAPHY

- CHAPIN, E. A. 1926 *Eustrongylides ignotus* Jägersk. in the United States. J. Parasitol. 13: 86-87.
- HUNTER, G. W. III. 1937 Parasitism of fishes in the lower Hudson area. Suppl. 26th. Ann. Rep. N. Y. St. Conserv. Dept. Biol. Surv. No. 11: 264-273.
- 1942 Studies on the parasites of fresh water fishes of Connecticut. Conn. St. Geol. Nat. Hist. Survey Bull. No. 63: 228-288.
- MUELLER, J. F. 1934 Additional studies on parasites of Oneida Lake fishes including descriptions of new species. Bull. N. Y. St. Coll. Forestry 7: 335-373.
- VON BRAND, T. 1938 Physiological observations on a larval *Eustrongylides* (Nematoda). J. Parasitol. 24: 445-451.
- 1944 Physiological observations on a larval *Eustrongylides*. VI. Transmission to various coldblooded intermediate hosts. Proc. Helminth. Soc. Washington 11: 23-27.
- AND SIMPSON, W. F. 1944 Physiological observations on a larval *Eustrongylides*. VII. Studies upon survival and metabolism in sterile surroundings. J. Parasitol. 30: 121-129.

## STUDIES OF SHEEP PARASITES. V. IMMUNITY TO GASTRO-INTESTINAL NEMATODES\*

PHILIP A. HAWKINS AND C. L. COLE

Michigan Agricultural Experiment Station

Stoll (1929) showed that sheep developed a marked resistance to *Haemonchus contortus* infections, as manifested by a drop in egg count in parasitized animals. Stoll and Nelson (1930) indicated that this resistance was humoral in nature by experiments in which saline extracts of this parasite produced an immediate marked intradermal reaction. Stumberg (1933) by an essentially anaphylactic test was able to detect antibodies of *H. contortus* at a dilution of 1:50,000. Studies since this time have been confined mainly to a confirmation of Stoll's (1929) work, not only for *H. contortus*, but for the other nematodes parasitic in the alimentary tract of sheep.

Sarles (1937, 1938) developed a new technique for the detection of antibody produced by helminth infections. He showed that when the sera of rats immune to *Nippostrongylus muris* were placed in contact with the preparasitic larvae, a precipitate was formed at various locations on, or in them. These reactions occurred principally about the mouth, excretory pore, cuticle and in the digestive tract. This precipitate decreased the activity of the larvae and inhibited their development. Similar reactions have since been noted by Otto (1940) in *Ancylostoma caninum*, Mauss (1940) and Oliver-González (1940) in *Trichinella spiralis* and Oliver-González (1943) in *Ascaris lumbricoides*.

### METHODS

The immune serum was obtained from three lambs and a ewe which had undergone a natural infection and recovery with *H. contortus*, *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *Cooperia curticei*, *Nematodirus* sp., *Oesophagostomum columbianum*, *Chabertia ovina* and *Trichuris ovis*. These sheep were kept in the same pasture and maintained under similar conditions. Methods of egg counting have been described by Hawkins et al (1944). The sheep were bled, the blood stored in a refrigerator overnight, and the serum separated the next day.

The larvae were obtained from the feces of lambs with mixed naturally acquired nematode infections. The feces were mixed with charcoal and cultured for two weeks at room temperatures. The larvae were isolated with a Baermann apparatus, and then washed repeatedly in sterile distilled water. They were allowed to stand overnight in sterile distilled water after which they were again washed. The larvae were then placed in 0.1% mercuric chloride for half an hour.

Lapage (1933) and Glaser and Stoll (1940) have shown that solutions of hypochlorite will exsheath strongyle larvae. In order to obtain exsheathed larvae the suspension was placed in 0.2% calcium hypochlorite for half an hour. The larvae were then washed several times in sterile distilled water.

---

Received for publication, January 9, 1945.

\* Journal Article No. 740 (n. s.) of the Michigan Agricultural Experiment Station, East Lansing.

For the tests the suspensions of larvae and sera were placed on a sterile slide, a cover glass placed on them and ringed with sterile petrolatum. The preparations were kept at 37° C and examined several times a day at both low and high magnification. The species of larvae in each test were not identified.

#### RESULTS AND DISCUSSION

The egg counts of the ewe and lambs for the summer months are given in Fig. 1. It will be noted that the ewe had a much lower level of parasitism than the lambs. This is characteristic as has been pointed out by Hawkins et al (1944). The resistance of mature sheep to parasitism is much greater than that of the lambs.

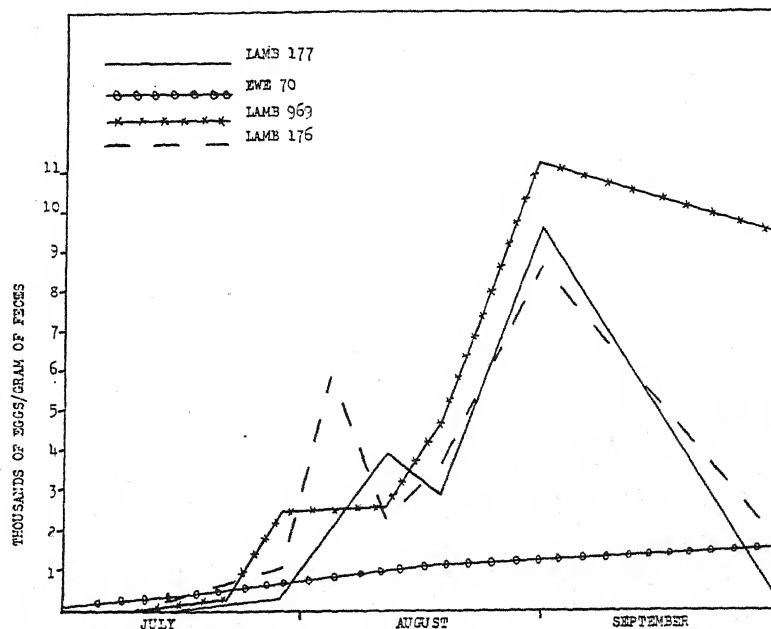


FIG. 1. Total egg counts.

The acquisition of parasitism by the three lambs was similar, although the elimination of the infection or the acquisition of resistance is quite different. Lamb 969 failed to throw off the infection as shown by an egg count of 9600 per gram of feces at the end of the experiment, September 29. Lamb 176 was more successful in ridding itself of parasites, having an egg count of 1800, while lamb 177 lost most of its roundworm parasites as shown by a total egg count of only 300. The effects of this resistance are shown by the weights of these lambs. Lamb 969 was 18% lighter than on July 14; lamb 176 was 4% heavier, and lamb 177 was 6% heavier. While none of these lambs had made economical gains, it was only in those lambs in which a resistance was developing that any weight gains were being made.

In an attempt to determine the nature of this resistance, a suspension of ensheathed nematode larvae was placed in sera of the above-mentioned ewe and lambs. These larvae consisted of *Haemonchus contortus* (68%), *Chabertia ovina* (14%), *Cooperia curticei* (12%), *Oesophagostomum columbianum* (4%) and *Trichostrongylus colubriformis* (2%). No abnormal appearance of the ensheathed larvae could be detected after two weeks' incubation at either 37° C or room temperature.



TABLE I

	Larvae with ppt	Oral ppt	Anal ppt	Excretory pore ppt	Cuticle ppt
Ewe 70 .....	% 78.0	% 76.0	% 26.1	% 18.2	% 22.0
Lamb 969 .....	45.3	41.5	7.5	0.0	1.8
Lamb 176 .....	48.5	48.5	6.1	0.0	0.0
Lamb 177 .....	88.0	88.0	16.6	9.5	2.3

However, it was noted that a precipitate formed about the mouth of a few of the larvae that had lost their sheaths.

Since it seemed apparent that a precipitate would not form about ensheathed larvae, the sera were next placed in suspensions of exsheathed larvae. In these suspensions a high percentage of the larvae had precipitates about either the mouth, excretory pore, anus or cuticle. The types of oral and anal precipitate are seen in Figs. 2 and 3. The results of this experiment are given in Table I. No precipitates were formed in similar suspensions of larvae placed in the sera of lambs that had been raised parasite free, except for coccidia or *Strongyloides*, or in saline solutions.

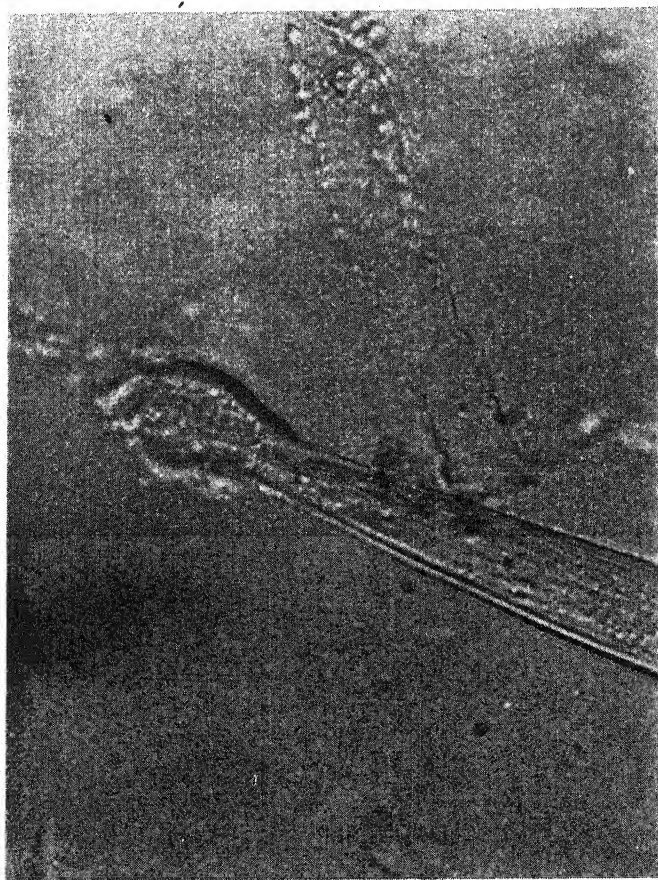


FIG. 2. Oral precipitate.  $\times 705$ .

It may be assumed that this is the type of reaction which enables sheep to throw off the greater part of their parasite burden. After a certain stage in the infection, antibodies are formed. These are capable of forming a precipitate around the mouth and excretory openings of the larvae. Since the severe effects of parasitism in sheep are largely due to the cumulative effects of constant reinfection, it is readily seen that the host may acquire an immunity which will prevent reinfection. Thus, when the larvae are embedded in the gastric or intestinal mucosa, they may be immobilized by this antibody and then the phagocytic cells of the body may destroy them. Such

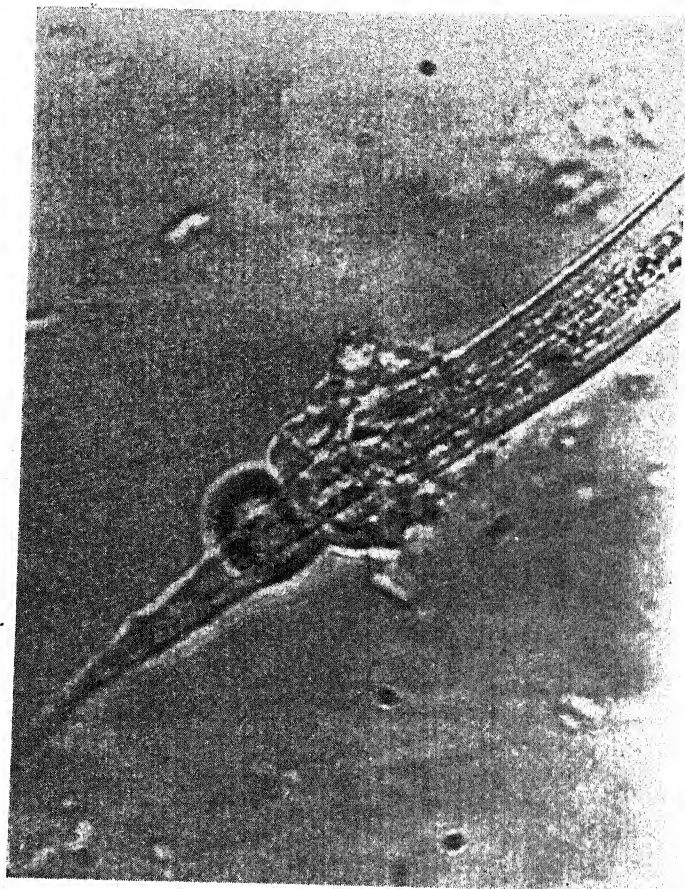


FIG. 3. Anal precipitate.  $\times 705$ .

a parallelism between the effects of immune sera *in vitro* and *in vivo* has been demonstrated by Taliaferro and Sarles (1939) in *Nippostrongylus muris* infections and by Oliver-González (1941) in *T. spiralis* infections.

It may be assumed that since lamb 177 had serum capable of forming the precipitate in a large percentage of the larvae *in vitro*, it should have a well-developed immunity. Such was the case as shown by its low egg count and its beginning to gain weight. Serum from lamb 969 formed a precipitate about slightly less than half of the larvae *in vitro*, and as evidenced by its egg count and weight had not developed any marked immunity to reinfection.

It might be presumed from inspection of the egg counts that ewe 70 and lamb 176 would have the same degree of immunity. However, it will be noted that ewe 70 had a relatively low level of infection during the summer, and from inspection of Table I it will be seen that the serum of this ewe, when placed in contact with larvae, produced a precipitate in 78% of the larvae. The ewe had a relatively high degree of immunity all summer. Lamb 176, however, was developing an immunity as indicated by its egg count in Fig. 1. This is shown by the fact that only 48.5% of the larvae placed in the immune serum of this lamb developed a precipitate. This relationship between egg counts at the time serum was obtained and the percentage of larvae reacting with the serum to produce a precipitate is shown in Fig. 4.

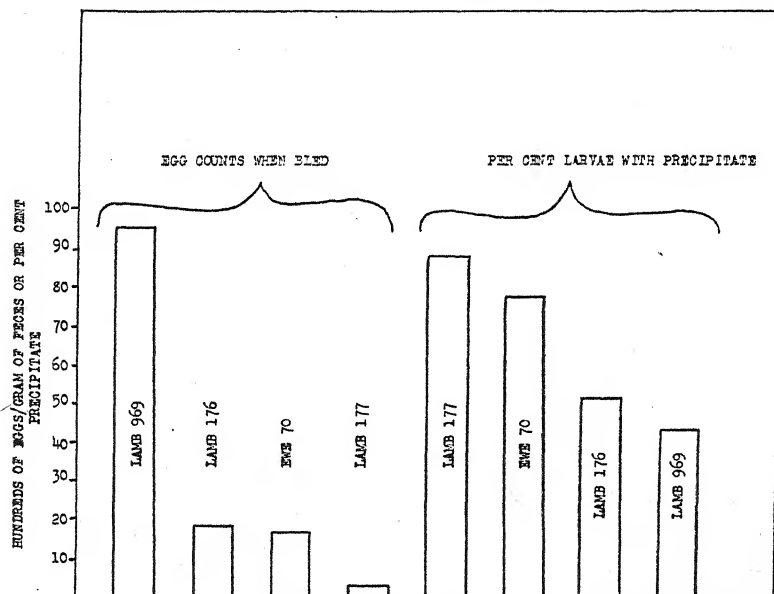


FIG. 4. Relationship between total egg counts and percentage of precipitate formed about larvae.

#### SUMMARY

Exsheathed strongyle larvae of sheep react to immune serum from naturally infected sheep by the formation of a precipitate in one or more of the following locations: (1) mouth, (2) anus, (3) excretory pore and/or (4) cuticle. The mechanism of immunity against the nematode parasites of the alimentary tract of sheep is postulated.

#### REFERENCES

- GLASER, R. W. AND STOLL, N. R. 1940 Exsheathing and sterilizing infective nematode larvae. *J. Parasitol.* 26: 87-94.
- HAWKINS, PHILIP A., COLE, C. L., KLINE, E. E. AND DRUDGE, J. H. 1944 Studies of sheep parasites. I. The course of untreated nematode infections. *Vet. Med.* 29: 154-161.
- LAPAGE, G. 1933 Cultivation of parasitic nematodes. *Nature* 131: 583.
- MAUSS, E. A. 1940 The *in vitro* effect of immune serum upon *Trichinella spiralis* larvae. *Amer. J. Hyg.* 32: 80-83.
- OLIVER-GONZÁLEZ, J. 1940 The *in vitro* action of immune serum on the larvae and adults of *Trichinella spiralis*. *J. Inf. Dis.* 67: 292-300.
- 1941 The dual antibody basis of acquired immunity in trichinosis. *J. Inf. Dis.* 69: 254-270.

- 1943 Antigenic analysis of the isolated tissues and body fluids of the roundworm *Ascaris lumbricoides* var. *suum*. J. Inf. Dis. 72: 202-212.
- OTTO, G. F. 1940 A serum antibody in dogs actively immunized against the hookworm, *Ancylostoma caninum*. Amer. J. Hyg. 31: 23-27.
- SARLES, M. P. 1937 The *in vitro* action of immune rat serum on *Nippostrongylus muris* (Nematoda). J. Parasitol. 23: 560-561.
- 1938 The *in vitro* action of immune rat serum on the nematode *Nippostrongylus muris*. J. Inf. Dis. 62: 337-348.
- STOLL, N. R. 1929 Studies with the stronggloid nematode *Haemonchus contortus*. I. Acquired resistance of hosts under natural reinfection conditions out of doors. Amer. J. Hyg. 10: 384-418.
- AND NELSON, J. B. 1930 Intradermal tests with *Haemonchus contortus* in sheep and goats. J. Parasitol. 17: 116.
- STUMBERG, J. E. 1933 The detection of proteins of the nematode *Haemonchus contortus* in the sera of infected sheep and goats. Amer. J. Hyg. 18: 247-265.
- TALIAFERRO, W. H. AND SARLES, M. P. 1939 The cellular reactions in the skin, lungs and intestine of normal and immune rats after infection with *Nippostrongylus muris*. J. Inf. Dis. 64: 157-192.



*PHLEBOTOMUS (DAMPFOMYIA) ANTHOPHORUS*, N. SP., AND  
*PHLEBOTOMUS DIABOLICUS* HALL FROM TEXAS  
(DIPTERA: PSYCHODIDAE)<sup>1</sup>

C. J. ADDIS

During the summer of 1944 a large number of sandflies of the genus *Phlebotomus* was collected at Uvalde, Texas, and brought to Houston in an effort to establish a laboratory colony. Although sandflies had been collected in and around human habitations during the latter part of May and early June, none was found in such localities during July, August or the first part of September. Towards the end of September sandflies were again obtained from human habitations. During the months of July, August and September sandflies were collected from a rabbit pen attached to the Field Experiment Station of the U. S. Bureau of Entomology and Plant Quarantine.

The specimens found in and around homes, biting human beings, were identified as *Phlebotomus diabolicus* Hall, 1936. Those collected from the rabbits proved to be a previously undescribed species possessing a number of unusual characteristics which warrant the erection of a new subgenus to include it, for which the name *Dampfomyia* is proposed. The specific name *anthophorus* is proposed for this new form, based on the peculiar characters of the spermatheca. A description of *Phlebotomus (Dampfomyia) anthophorus*, and an amended description of *Phlebotomus diabolicus* are given below.

*Phlebotomus (Dampfomyia) anthophorus*, n. sp.

*Phlebotomus (Dampfomyia) anthophorus* was first observed by Mr. H. M. Brundrett of the Bureau of Entomology and Plant Quarantine in June feeding on rabbits at the Experiment Station in the northern and more elevated part of Uvalde. Observations on these flies were made by the writer at intervals from July to September. The females were found feeding on the rabbits and resting in the vicinity only in the mornings; no males were ever seen. On some mornings up to a hundred flies were collected between 8 and 10 AM, with very few being observed after noon. None were ever seen prior to sunrise or after sundown. The flies ceased to appear late in November, and were not again observed until March 23, 1945.

The rabbit pens where the flies were collected occupied a room attached to the north side of the building and was shaded practically all day. The room was screened on three sides, covered by a roof and provided with a concrete floor. Four or five rabbits were kept separately or in pairs in three large boxes made of wooden slats, with manure pans under the floors. These pans were cleaned only at intervals of several weeks and were thought to be possible breeding places for the flies, but no

---

Received for publication, January 17, 1945.

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Rice Institute, Houston, Texas. The author wishes to express his sincere appreciation to Dr. Asa C. Chandler for his advice and aid in carrying out this work, to Mrs. Evelyn Hake for the preparation of the slides and to Mr. and Mrs. H. M. Brundrett for their aid in collecting the specimens.



larvae were found, and the absence of any males suggests that the breeding occurred outside the shed.

In order to determine the distribution of this sandfly in Uvalde, observations were made on other rabbits, dogs, cattle, horses, and goats throughout the city, but no *Phlebotomus* was found, even on penned rabbits kept within 50 yards of the Station. Dr. A. Dampf reports (unpublished) collection of a single female specimen, on September 24, 1937, from a house in Cuantla, in the State of Morelos, south of Mexico City.

The flies refused to feed on white rats placed in cages adjoining the rabbits. Attempts to feed them on a human arm in the laboratory at Houston were unsuccessful, but they fed readily on Syrian hamsters. The flies had the characteristic *Phlebotomus* habit of making short hops when disturbed, and were very easily collected.

In trying to locate the breeding places, several caged rabbits were placed by rock piles, old tree stumps, wood rat and ground squirrel nests, goat pens and chicken houses within a radius of a hundred yards of the Station, and in similar locations on the South side of the city. No *Phlebotomus* were found on these rabbits either during the morning or after sundown.

In summary, *P. anthophorus* appears to feed only in the morning, never having been found after sundown. It has a definite preference for rabbit blood but will feed on hamsters in the laboratory. It is apparently not annoying to man since attempts to feed it on human blood have failed, and, except for the single specimen collected by Dampf in Mexico, has not been observed in houses.

Captured females brought back to Houston laid eggs, from which several males and females have developed. A detailed account of the life cycle will be given in a subsequent paper.

Subgenus *Dampfomyia*, new subgenus

*Male*: Segment V of palpi long, longer than any of the other segments. Geniculate spines of antennae without posterior "spur," extending to distal end of their respective segments, and inserted on proximal one-third except in segment III, where inserted on distal one-third. Genitalia with basal segment of upper clasper without tuft of setae. Distal segment with three spines; one apical, one median and ventral, and one on inner side between apical and median spines. Median clasper with setae on distal end, and bearing a large appendage attached to its upper surface and ending distally in a saucer-shaped expansion bearing a number of setae on its margin. Lower clasper longer than median clasper or basal segment of upper clasper.

*Female*: Palpi and antennae as in male. Anterior pharynx with lateral teeth on each side; posterior border of floor forming a smooth curve, with a pair of laterally-attached, club-shaped appendages and a chitinized bar on its upper surface. Spermatheca with a lobated, rosette-like expansion around head, followed posteriorly by an enlarged, oval structure which

PLATE I

*Phlebotomus (Dampfomyia) anthophorus*, n. sp.

- FIG. 1. Antennal segments showing geniculate spines.
- FIG. 2. Palpus.
- FIG. 3. Side view of male genitalia.
- FIG. 4. Head.
- FIG. 5. Anterior pharynx.
- FIG. 6. Spicular apparatus, genital pump and part of spicules.
- FIG. 7. Wing.
- FIG. 8. Side view of female genitalia.
- FIG. 9. Spermatheca (lobated expansion around head somewhat collapsed due to preparation).

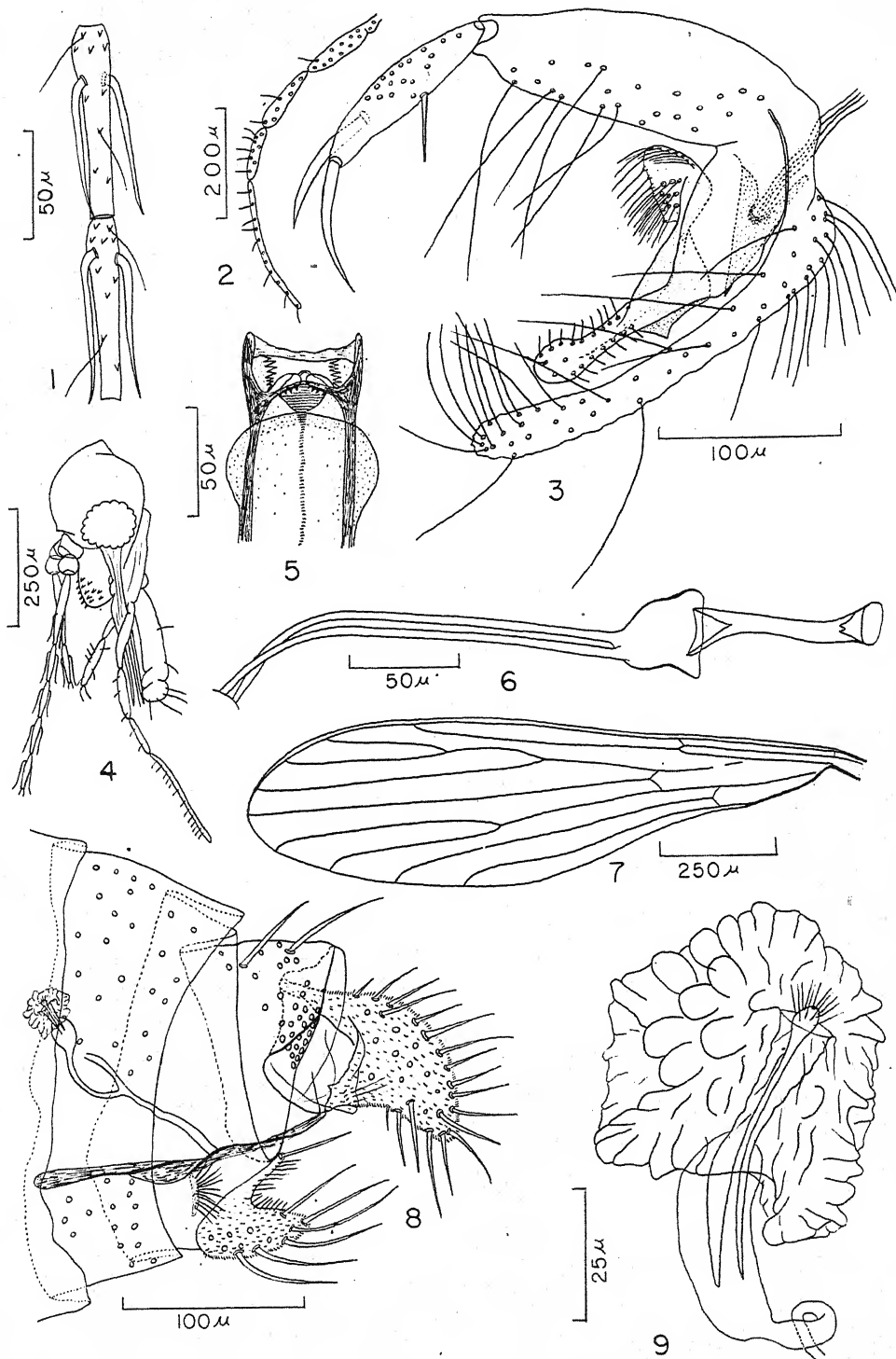


PLATE I

abruptly narrows to form the duct. Ducts fine and membranous, emptying into a narrow common duct.

*Larva*: Four caudal setae in second to fourth instars.

*Phlebotomus anthophorus*, n. sp.<sup>2</sup>

**MALE**: About 1.3 mm in length; yellowish in color.

*Head*: Clypeus 116-124 (119) microns in length, longer than the greatest diameter of eyes.

*Epipharynx*: 177-194 (182) microns in length, measured from anterior border of clypeus.

*Palpi*: 551-600 (575) microns in length. Measurement of individual segments as follows: I=28-31 (29) microns, II=87-104 (98) microns, III=121-134 (124) microns, IV=83-101 (89) microns, V=219-239 (234) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 3.2 \quad \begin{array}{l} V > II + III; V > III + IV; \\ V < II + III + IV. \end{array}$$

*Antennae*: Geniculate spines without posterior "spur," extending to distal end of their respective segments, and inserted on proximal one-third except in segment III, where inserted on distal one-third. Measurements of individual segments as follows: III=174-198 (185) microns, IV=101-104 (103) microns, V=101-104 (103) microns, VI=97-104 (101) microns, VII=97-101 (99) microns, VIII=94-100 (98) microns, IX=94-97 (96) microns, X=87-97 (93) microns, XI=87-94 (90) microns, XII=83-90 (86) microns, XIII=76-87 (82) microns, XIV=66-80 (73) microns, XV=62-69 (64) microns, XVI=59-62 (60) microns.

$$III < IV + V; III < XII - XVI; IV + V + VI < XII - XVI.$$

$$\frac{III}{\text{epipharynx}} = 1.0 \quad \text{Antennal formula} = \frac{2}{III - XV}$$

*Thorax*: Mesonotum and scutellum yellowish brown, pleura yellowish.

*Wings*: Long and narrow, 1220-1317 (1256) microns in length and 343-373 (362) microns in greatest width. Measurements of wing veins as follows: alpha=224-239 (233) microns, beta=209-216 (213) microns, gamma=239-254 (248) microns, delta=52-60 (56) microns.

$$\frac{\text{length}}{\text{width}} = 3.5 \quad \frac{\alpha}{\beta} = 1.1 \quad \frac{\alpha}{\gamma} = 0.94 \quad \frac{\alpha}{\delta} = 4.2$$

*Legs*: No striking characters.

*Forelegs*: Femur=561-582 (573) microns, tibia=548-561 (556) microns, tarsal segments: I=287-313 (303) microns, II=168-179 (171) microns, III=104-122 (118) microns, IV=89-104 (101) microns, V=60-75 (70) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 0.97 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

*Midlegs*: Femur=582-604 (592) microns, tibia=686-722 (702) microns, tarsal segments: I=358-373 (365) microns, II=179-194 (187) microns, III=119-134 (127) microns, IV=96-104 (101) microns, V=75-81 (79) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.2 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 2.0$$

*Hindlegs*: Femur=627-658 (639) microns, tibia=1000-1114 (1028) microns, tarsal segments: I=432-454 (441) microns, II=224-243 (233) microns, III=134-152 (143) microns, IV=104-121 (112) microns, V=75-89 (82) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.6 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.9$$

<sup>2</sup> Measurements based on a study of six flies, average given in parenthesis.

PLATE II

*Phlebotomus diabolicus* Hall

- FIG. 10. Spicular apparatus, genital pump and part of spicules.
- FIG. 11. Palpus.
- FIG. 12. Side view of male genitalia.
- FIG. 13. Antennal segments showing geniculate spines.
- FIG. 14. Anterior pharynx.
- FIG. 15. Wing.
- FIG. 16. Side view of female genitalia.
- FIG. 17. Spermatheca.
- FIG. 18. Head.

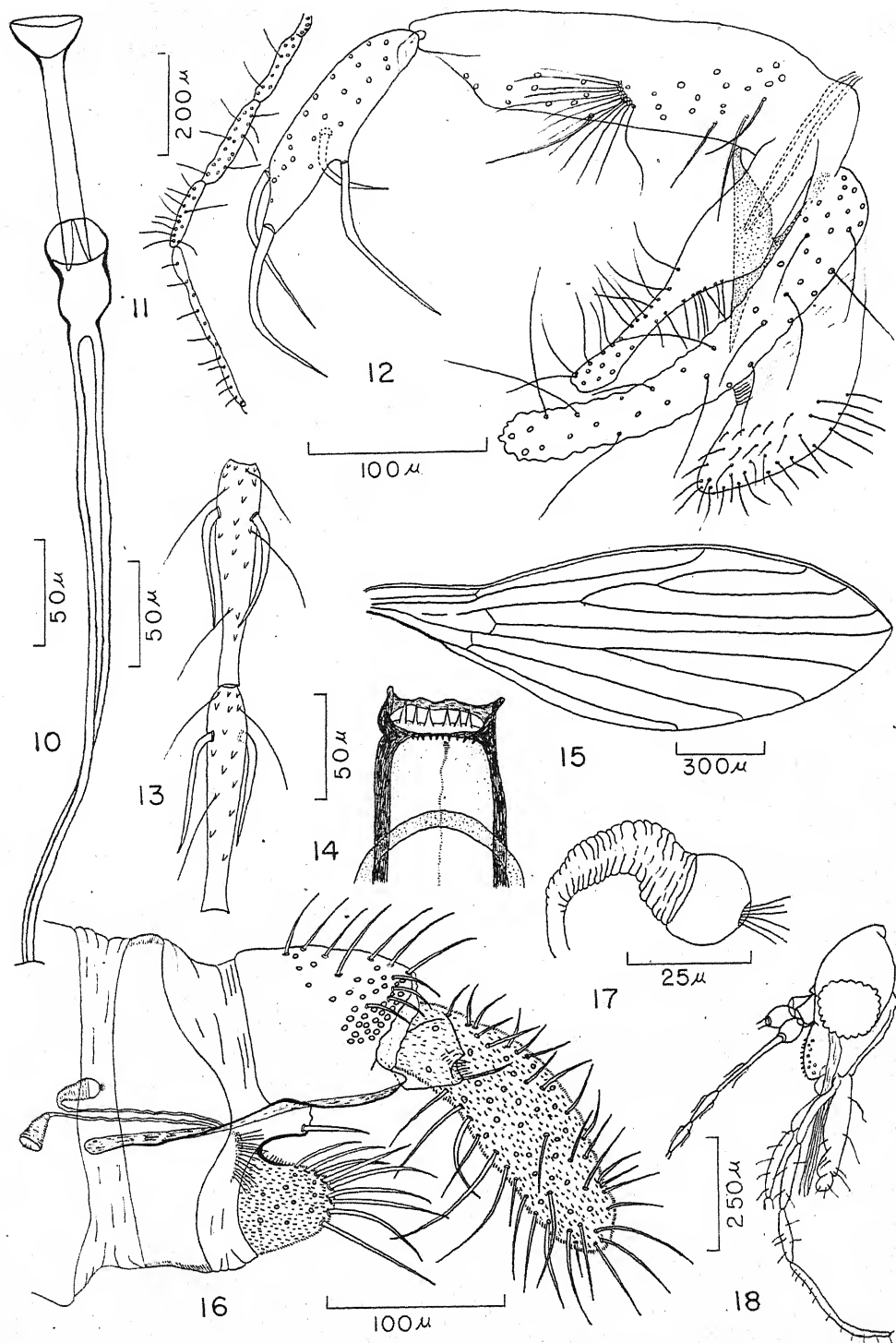


PLATE II

*Abdomen:* No striking characters.

*Genitalia* (Plate I, Fig. 3): Basal segment of upper clasper 179–209 (191) microns in length, with no tuft of setae. Distal segment 118–125 (120) microns in length, with three spines arranged in following manner: one apical; one median and ventral, finer than apical one; and one, similar to apical spine, inserted on inner side midway between apical and median spines.

Median clasper: 149–198 (174) microns in length, with fine setae and with an inner appendage, attached along middle third of dorsal side of clasper, and terminating in a saucer-shaped expansion with about 25 setae arranged around margin.

Lower clasper: 254–274 (259) microns in length, definitely longer than median clasper or basal segment of upper clasper.

Spicular apparatus (Plate I, Fig. 6): genital pump 101–111 (105) microns in length, spicules 288–303 (294) microns in length.

*FEMALE:* Larger than male (about 2 mm in length); coloration as in male.

*Head* (Plate I, Fig. 4): Similar to male, clypeus 125–149 (141) microns in length.

*Epipharynx:* 209–239 (225) microns in length, measured from anterior border of clypeus.

*Palpi* (Plate I, Fig. 2): 524–591 (570) microns in length. Measurements of individual segments as follows: I=31–35 (34) microns, II=83–104 (95) microns, III=115–132 (127) microns, IV=83–101 (94) microns, V=208–232 (220) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 2.5 \quad \begin{array}{l} V \pm II + III; V \pm III + IV; \\ V < II + III + IV. \end{array}$$

*Antennae* (Plate I, Fig. 1): Geniculate spines as in male. Measurements of antennal segments as follows: III=146–180 (163) microns, IV=73–87 (79) microns, V=73–87 (79) microns, VI=73–87 (79) microns, VII=73–87 (79) microns, VIII=73–87 (79) microns, IX=73–85 (78) microns, X=73–83 (78) microns, XI=73–80 (77) microns, XII=69–76 (73) microns, XIII=66–73 (69) microns, XIV=52–62 (59) microns, XV=45–52 (49) microns, XVI=38–49 (45) microns.

$$\begin{array}{l} III > IV + V; III < IV + V + VI; III < XII - XVI; \\ IV + V + VI < XII - XVI. \end{array}$$

$$\frac{III}{\text{epipharynx}} = 0.72 \quad \text{antennal formula} = \frac{2}{III - XV}$$

*Anterior pharynx* (Plate I, Fig. 5): No horizontal teeth but with about seven lateral teeth on each side. Posterior border of floor forming a smooth curve, and with a pair of laterally attached, club-shaped appendages and a chitinated bar on its upper surface. Vertical teeth few (about 8 in number) and small. Pigmented area triangular in shape and very rough at posterior end, followed by a long and narrow area.

*Posterior pharynx:* No striking characters.

*Thorax:* As in male.

*Wings* (Plate I, Fig. 7): 1463–1524 (1494) microns in length and 433–507 (459) microns in greatest width. Wing veins with measurements as follows:  $\alpha$ =298–373 (333) microns,  $\beta$ =224–269 (242) microns,  $\gamma$ =269–298 (291) microns,  $\delta$ =63–149 (116) microns.

$$\frac{\text{length}}{\text{width}} = 3.3 \quad \frac{\alpha}{\beta} = 1.4 \quad \frac{\alpha}{\gamma} = 1.1 \quad \frac{\alpha}{\delta} = 2.0$$

*Legs:* No striking characters.

*Forelegs:* Femur=522–597 (545) microns, tibia=494–522 (515) microns, tarsal segments: I=283–328 (301) microns, II=164–179 (171) microns, III=104–119 (117) microns, IV=90–104 (92) microns, V=60–75 (73) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 0.94 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

*Midlegs:* Femur=537–627 (567) microns, tibia=598–656 (622) microns, tarsal segments: I=328–388 (351) microns, II=179–224 (194) microns, III=119–149 (134) microns, IV=90–119 (104) microns, V=60–75 (73) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.1 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

*Hindlegs:* femur=612–686 (642) microns, tibia=821–910 (863) microns, tarsal segments: I=462–492 (473) microns, II=209–239 (229) microns, III=134–149 (144) microns, IV=104–119 (112) microns, V=60–75 (73) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.3 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 2.1$$



*Abdomen*: with erect bristles.

*Spermatheca* (Plate I, Figs. 8 and 9): With a lobated, rosette-shaped expansion around head; head small with fine setae, followed posteriorly by an enlarged oval structure which narrows abruptly to form the duct. Ducts fine and with smooth membranous walls, emptying into a narrow common duct.

*Type material*. *Holotype*: Male (stained whole mount); reared in the biological laboratories of the Rice Institute by C. J. Addis during the summer of 1944 from eggs laid by females collected in Uvalde, Texas, July, 1944. To be deposited in the United States National Museum.

*Allotype*: Female (stained dissected mount); collected by C. J. Addis at Uvalde, Texas, July, 1944. To be deposited in the United States National Museum.

*Paratypes*: Several females collected with the allotype and several males reared in the laboratory with the holotype. To be deposited in the collections of Asa C. Chandler and C. J. Addis.

*Taxonomic discussion*: See below *Phlebotomus diabolicus*.

### *Phlebotomus diabolicus* Hall, 1936

Hall (1936) described *Phlebotomus diabolicus* from specimens captured at Uvalde, and Lindquist (1936) published notes on its habits and biology. The observations of the writer corroborate those of Hall and Lindquist. During the past season specimens of *P. diabolicus* were first noticed in the houses at night during the latter part of May. They disappeared toward the middle of June and did not reappear until the last of September. During October and up to November 16, when the writer received the last shipment of specimens from Uvalde, the flies could be caught in fair numbers at night under the lights in houses and on lawns. Females shipped to the laboratory in Houston readily fed on a human arm, and some took blood meals from Syrian hamsters. Eggs laid in the laboratory failed to develop beyond the fourth larval stage.

*MALE*: 2 mm in length; blackish-brown in color with silvery sheen.

*Head*: Clypeus 142–149 (146) microns in length, shorter than greatest diameter of eyes.

*Epipharynx*: 240–264 (255) microns in length, measured from anterior border of clypeus.

*Palpi*: 634–721 (673) microns in length. Measurements of individual segments as follows: I = 28–35 (31) microns, II = 121–142 (131) microns, III = 153–173 (163) microns, IV = 104–125 (113) microns, V = 232–246 (240) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 2.6 \quad \begin{array}{l} V < II + III; \\ V < III + IV; \\ V < II + III + IV. \end{array}$$

*Antennae*: geniculate spines without posterior "spur," extending about three-fourths the distance to distal end of their respective segments, and inserted on proximal one-third except in segment III, where inserted on distal one-third. Measurements of individual segments as follows: III = 222–249 (237) microns, IV = 101–118 (108) microns, V = 101–118 (108) microns, VI = 97–115 (106) microns, VII = 97–115 (104) microns, VIII = 94–111 (101) microns, IX = 94–101 (97) microns, X = 90–97 (95) microns, XI = 87–94 (92) microns, XII = 83–90 (86) microns, XIII = 76–83 (79) microns, XIV = 59–66 (63) microns, XV = 56–59 (57) microns, XVI = 49–52 (51) microns.

$$\begin{array}{l} III > IV + V; \quad III > IV + V + VI; \quad III > XII - XVI; \\ IV + V + VI > XII - XVI. \end{array}$$

$$\frac{III}{\text{epipharynx}} = 0.93 \quad \text{antennal formula} = \frac{2}{III - XV}$$

*Thorax*: Mesonotum and scutellum blackish-brown, pleura lighter.

*Wings*: Bluntly lanceolate, 1854–1902 (1872) microns in length and 627–642 (634) microns in greatest width. Measurements of wing veins as follows: alpha = 448–470 (459) microns, beta = 245–283 (265) microns, gamma = 358–388 (367) microns, delta = 104–134 (112) microns.

$$\frac{\text{length}}{\text{width}} = 3.0 \quad \frac{\alpha}{\beta} = 1.7 \quad \frac{\alpha}{\gamma} = 1.3 \quad \frac{\alpha}{\delta} = 4.1$$

*Legs*: no striking characters.

*Forelegs*: Femur = 716–731 (724) microns, tibia = 731–754 (742) microns, tarsal segments: I = 373–418 (398) microns, II = 209–239 (226) microns, III = 130–149 (138) microns, IV = 119–134 (125) microns, V = 75–90 (86) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.0 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

Midlegs: Femur = 731-761 (748) microns, tibia = 878-910 (888) microns, tarsal segments: I = 442-467 (453) microns, II = 239-254 (245) microns, III = 138-164 (153) microns, IV = 119-128 (121) microns, V = 86-93 (89) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.2 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.8$$

Hindlegs: Femur = 791-836 (819) microns, tibia = 1119-1149 (1135) microns, tarsal segments: I = 566-612 (592) microns, II = 283-303 (291) microns, III = 179-194 (183) microns, IV = 134-149 (145) microns, V = 90-104 (95) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.4 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 2.0$$

*Abdomen*: No striking characters.

*Genitalia* (Plate II, Fig. 12): Basal segment of upper clasper 194-236 (213) microns in length, with a well-defined tuft of setae on inner side. Distal segment 148-163 (156) microns in length, with four long spines arranged in the following manner: one apical; one median and ventral; one median on inner side; and one dorsal, midway between apical and median spines.

Median clasper: 164-184 (177) microns in length.

Lower clasper: 205-246 (233) microns in length, longer than median clasper or basal segment of upper clasper.

Spicular apparatus (Plate II, Fig. 10): Genital pump 135-153 (142) microns in length, spicules 388-412 (399) microns in length.

*FEMALE*: 2 mm in length, coloration as in male.

*Head* (Plate II, Fig. 18): Similar to male, clypeus 146-153 (151) microns in length.

*Epipharynx*: 269-298 (281) microns in length, measured from anterior border of clypeus.

*Palpi* (Plate II, Fig. 11): 792-865 (838) microns in length. Measurements of individual segments as follows: I = 31-42 (38) microns, II = 139-156 (149) microns, III = 167-184 (179) microns, IV = 118-139 (132) microns, V = 330-347 (339) microns. Palpal formula: 1. 4. 2. 3. 5.

$$\frac{\text{palpus}}{\text{epipharynx}} = 3.0 \quad \begin{array}{l} V > II + III; V > III + IV; \\ V < II + III + IV. \end{array}$$

*Antennae* (Plate II, Fig. 13): Geniculate spines as in male. Measurements of antennal segments as follows: III = 226-243 (235) microns, IV = 101-104 (103) microns, V = 101-104 (103) microns, VI = 97-104 (101) microns, VII = 97-101 (99) microns, VIII = 94-101 (97) microns, IX = 90-101 (96) microns, X = 87-97 (93) microns, XI = 79-94 (89) microns, XII = 76-90 (86) microns, XIII = 73-87 (80) microns, XIV = 69-76 (73) microns, XV = 59-69 (64) microns, XVI = 59-62 (60) microns.

$$\begin{array}{l} III > IV + V; III < IV + V + VI; III < XII - XVI; \\ IV + V + VI < XII - XVI. \end{array}$$

$$\frac{III}{\text{epipharynx}} = 0.83 \quad \text{antennal formula} = \frac{2}{III - XV}$$

*Anterior pharynx* (Plate II, Fig. 14): With six horizontal teeth. Posterior border of floor forming a smooth curve, with a chitinated bar on its upper surface. Vertical teeth few and small. Pigmented area long and narrow.

*Posterior pharynx*: No striking characters.

*Thorax*: As in male.

*Wings* (Plate II, Fig. 15): 1805-2000 (1927) microns in length and 627-686 (669) microns in greatest width. Measurements of wing veins as follows: alpha = 537-582 (565) microns, beta = 254-269 (259) microns, gamma = 336-343 (341) microns, delta = 164-179 (172) microns.

$$\frac{\text{length}}{\text{width}} = 2.9 \quad \frac{\alpha}{\beta} = 2.2 \quad \frac{\alpha}{\gamma} = 1.7 \quad \frac{\alpha}{\delta} = 3.3$$

*Legs*: No striking characters.

Forelegs: Femur = 716-746 (731) microns, tibia = 776-806 (791) microns, tarsal segments: I = 388-418 (400) microns, II = 224-242 (236) microns, III = 145-149 (147) microns, IV = 119-129 (123) microns, V = 90-93 (92) microns.

$$\text{femur} > \text{tarsus I} \quad \frac{\text{tibia}}{\text{femur}} = 1.1 \quad \frac{\text{tarsus I}}{\text{tarsus II}} = 1.7$$

Midlegs: Femur = 746-776 (762) microns, tibia = 880-910 (890) microns, tarsal segments: I = 448-477 (467) microns, II = 239-254 (247) microns, III = 164-179 (168) microns, IV = 134-143 (137) microns, V = 90-93 (92) microns.

$$\begin{array}{lcl} \text{femur} > \text{tarsus I} & \frac{\text{tibia}}{\text{femur}} = 1.2 & \frac{\text{tarsus I}}{\text{tarsus II}} = 1.9 \end{array}$$

Hindlegs: Femur=836-880 (859) microns, tibia=1179-1268 (1209) microns, tarsal segments: I=612-642 (628) microns, II=298-328 (311) microns, III=169-194 (179) microns, IV=149-164 (154) microns, V=90-104 (95) microns.

$$\begin{array}{lcl} \text{femur} > \text{tarsus I} & \frac{\text{tibia}}{\text{femur}} = 1.2 & \frac{\text{tarsus I}}{\text{tarsus II}} = 2.0 \end{array}$$

*Abdomen:* With erect bristles.

*Spermatheca* (Plate II, Figs. 16 and 17): With smooth globular head, followed posteriorly by a rugose area. Ducts fine, with smooth membranous walls, and without a common duct.

*Material examined:* Six males and six females from Uvalde, Texas, collected by Mr. and Mrs. H. M. Brundrett, October and November, 1944.

This material was compared with the female allotype captured by A. W. Lindquist at Uvalde, Texas, November 14, 1934, and also with the description and drawing of a male by Hall (1936), and found to be specifically identical.

*Taxonomic discussion:* The addition of *Phlebotomus* (*Dampfomyia*) *anthophorus* makes a total of six known species of *Phlebotomus* in the United States: *P. vexator* Coquillett, which has been taken throughout the southern states, from Maryland to California; *P. diabolicus* Hall, from Texas; *P. texanus* Dampf, from Texas; *P. stewarti* Mangabeira and Galindo, from California; *P. limai* Fonseca, with a wide distribution in southern United States, having been taken in Alabama, Mississippi and North Carolina (Rozeboom, 1944); and *P. anthophorus*, from Texas. Specimens of *Phlebotomus* have been collected in Florida also, but have not been specifically identified.

*P. anthophorus* can readily be distinguished from the other known species of *Phlebotomus* in the United States by the appendage on the median clasper of the male genitalia, the peculiar characters of the anterior pharynx, and the flower-like spermathecae of the female.

#### REFERENCES

- HALL, D. G. 1936 *Phlebotomus* (*Brumptomyia*) *diabolicus*, a new biting gnat from Texas (Diptera: Psychodidae). Proc. Ent. Soc. Wash. 38: 27-29.
- LINDQUIST, A. W. 1936 Notes on the habits and biology of a sand fly *Phlebotomus diabolicus* Hall, in southwestern Texas (Diptera: Psychodidae). Proc. Ent. Soc. Wash. 38: 29-32.
- MANGABEIRA, FILHO, O. AND GALINDO, PEDRO 1944 The genus *Flebotomus* in California. Amer. Jour. Hyg. 40: 182-198.
- ROZEBOOM, L. E. 1944 *Phlebotomus limai* Fonseca in the United States (Diptera: Psychodidae). Jour. Parasit. 30: 274-275.

A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS  
*POLYMORPHUS* FROM THE AMERICAN COOT

HARLEY J. VAN CLEAVE

University of Illinois, Urbana

For many years the writer has been accumulating specimens of an unidentified acanthocephalan from the American coot (*Fulica americana*). Most of the specimens have been obtained as the result of routine field investigations of food habits of the birds. Until very recently all but two of the individuals have lacked the proboscis, since that organ had been broken off upon removal of the worms from the host intestine. The two complete individuals, both females, were collected by Dr. Justus F. Mueller from a coot on Oneida Lake in New York state. The proboscis on each of these two individuals was so distinctively different from that known for any other species that the slides were put aside in the belief that the shapes might be due to some procedure in handling or in killing the parasites. In November of 1944, the writer received from Mr. Robert Rausch a consignment of unidentified ACANTHOCEPHALA. Among these were specimens from six individuals of *Fulica americana* taken on Buckeye Lake in Ohio on November 11, 1943. Many of these specimens had been carefully dissected out from the host intestine and of these all of the females bore an inflated proboscis, of pear-like shape, specifically unlike that of any described species but identical with the two individuals from Oneida Lake. The enlarged, bulbous proboscis explains the readiness with which the anchored proboscis becomes broken from the neck when an attempt is made to dislodge the female worms from the host intestine. A new species of *Polymorphus* recognized for these forms is here described under the name *Polymorphus trochus*.

*Polymorphus trochus* n. sp.

(Figs. 1-5)

With the characteristics of the genus *Polymorphus*, modified from Meyer (1932) to include his genus *Profilicollis*. Body of females from 4.5 to 7.8 mm long; males, about 3.8 mm. Greatest diameter slightly back of the front end of the body-proper, gradually tapering posteriorly to a narrowed posterior extremity and anteriorly rounding rapidly to meet a relatively narrow neck. Swollen trunk region set with numerous cuticular spines. The neck, spineless, sub-cylindrical to barrel-shaped, slightly reduced in diameter at both ends, is from 0.4 to 1.1 mm long and from 0.35 to 0.54 mm in maximum diameter. Proboscis in female pear-shaped, much inflated at base, with a short, greatly reduced nipple-like termination. Proboscis of females about 0.38 to 0.56 mm long; from 0.30 to 0.49 mm in maximum diameter with reduced terminal portion 0.100 to 0.175 mm in diameter. Proboscis of observed males, without conspicuous inflation, about 0.385 to 0.560 mm long; 0.210 mm in maximum diameter and 0.140 mm in terminal portion. Most of the hooks near the middle zone of the proboscis about 35  $\mu$  long, many at both ends somewhat shorter (near anterior extremity 21 to 42  $\mu$  long; on basal region 21 to 35  $\mu$ ).

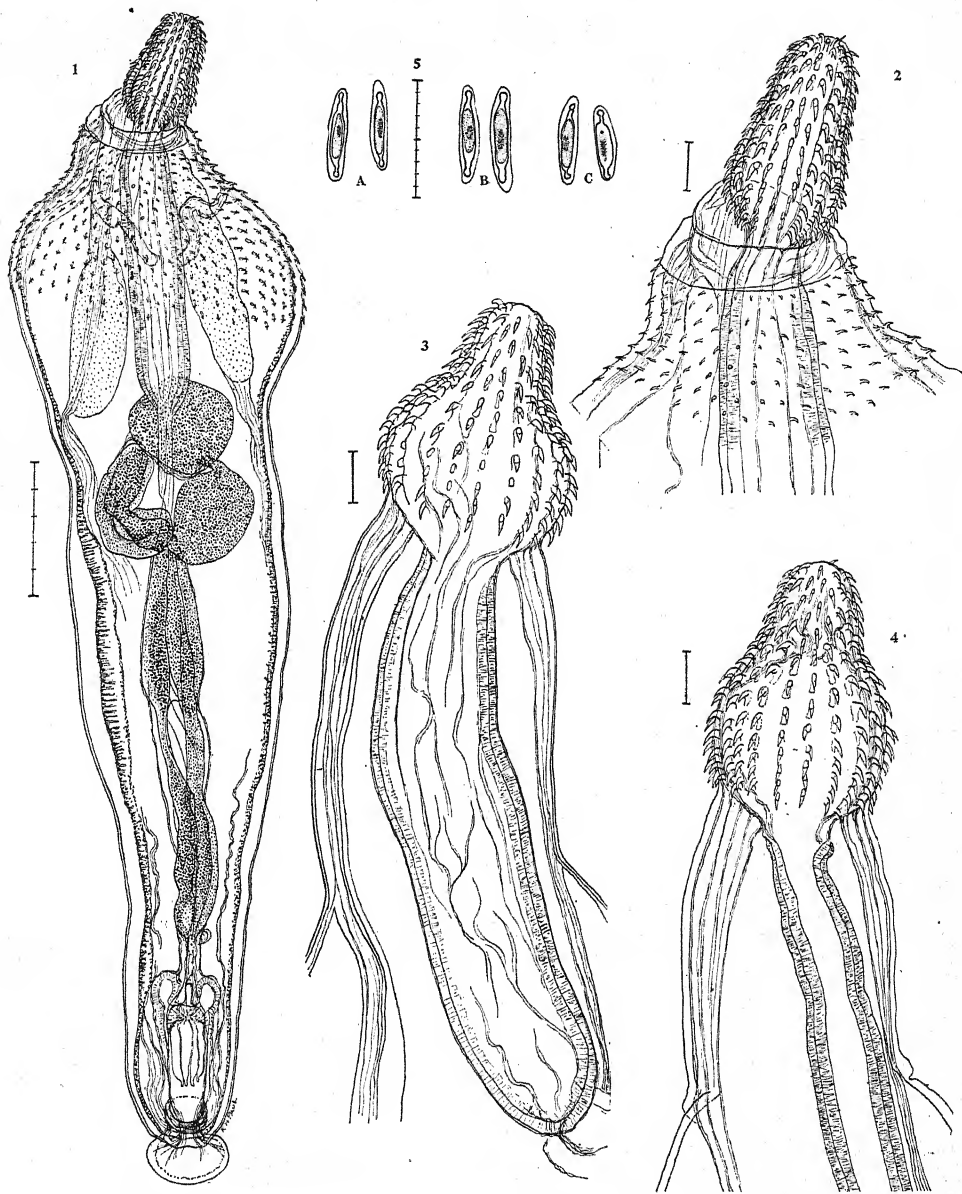
Embryos within body of mature females 75 to 84  $\mu$  by 14 to 20  $\mu$ .

*Host*: In intestine of American coot (*Fulica americana*) of Buckeye Lake, Ohio; Oneida Lake, New York; and Illinois River in Illinois.

*Holotype*: Female No. VC 3787.2; allotype male No. VC 3789.2 and series of paratypes (13 females and 1 male) in collection of H. J. Van Cleave, Urbana, Illinois.

In the past the line of demarcation between the genera *Polymorphus* and *Filicollis* has been misinterpreted often. Species now thought of as belonging to

Received for publication, February 27, 1945.



EXPLANATION OF PLATE  
*Polymorphus trochus* new species

All drawings were made with a camera lucida from stained permanent mounts in Damar. The writer is indebted to Mrs. Katharine Hill Paul for preparation of the drawings. The scale indicating magnification beside Fig. 1 has the value of 0.5 mm; all other scales on this plate have the value of 0.1 mm.

FIG. 1. Drawing of entire male (allotype VC 3789.2). The four cement glands are somewhat twisted and contorted in the region to the left of the testes. Note the two retinacula emerging from the wall of the proboscis receptacle anterior to the middle of that organ.

FIG. 2. Details of proboscis and anterior region of trunk in allotype male. Note that neck region is partially retracted within the body.

FIG. 3. Anterior extremity of a paratype female, showing the characteristic proboscis of the female of this species and its relation to the neck and receptacle of the proboscis.

FIG. 4. Proboscis and neck of holotype female (VC 3787.2).

FIG. 5. Three groups of embryos from different parts of the body cavity of a gravid female: A, from neck region; B, from middle of body; C, from near posterior extremity.



one of these genera have been mistakenly ascribed to the other because the importance of certain characteristics has been underestimated or ignored. At times this has resulted in the attempt to create new intermediate genera or to emend the generic diagnoses so as to include the intermediate forms in one of the genera already recognized. Thus in 1916 the writer suggested (page 132) that the inflated form of the proboscis, which some earlier workers had presumably shown to be a character appearing late in ontogeny, might be omitted from the list of diagnostic characters for the genus *Filicollis*. By a series of gradual steps in the literature, the species originally described as *Filicollis botulus* and *F. arcticus* were eliminated from *Filicollis* (see Meyer, 1931, Witenberg, 1932, and Van Cleave, 1939) and came to rest in the genus *Polymorphus*. This line of action took place in spite of the fact that at least several research workers had come to the conclusion that form of the inflated proboscis, regardless of the pattern of distribution of the proboscis hooks, was a character restricted to the genus *Filicollis* among the genera of avian POLYMORPHINAE.

Consequently, when the new species described in this paper as *P. trochus* was first studied, the swollen proboscis of the female seemed to call for assignment to *Filicollis*.

More recently, the writer has been restudying the problem of generic characters for the separation of the genera *Filicollis* and *Polymorphus* and has come to the conclusion that the distinctive arrangement and limitation of the hooks to the anterior face of the inflated, spheroidal proboscis is likewise distinctive of the genus *Filicollis*. These features of hook arrangement were so diverse from those found on the genotype, *Filicollis anatis*, that other features such as the form of the neck gave support to the proposal for including the species *trochus* within the genus *Polymorphus*.

*P. trochus* differs from all other species assigned to *Polymorphus* in the distinctive form of the proboscis. Though the proboscides of females of *P. botulus* and *P. arcticus*, are inflated and spheroidal at the end of an attenuated neck, the inflated proboscis of the female of *P. trochus* carries a terminal portion much reduced in size, projecting nipple-like from the inflated zone.

The very small numbers of males in the type material of *P. trochus* remains unexplained. The relatively smaller size of the male proboscis, lacking the bulbular enlargement, would be a factor predisposing toward recovery of a greater percentage of entire males than of females from the host intestine. Yet in the available collections there are 16 individuals with body and proboscis perfect enough to leave no doubt as to specific identity. Among these 16 individuals, all but two are female.

#### REFERENCES

- MEYER, A. 1931 Die Acanthocephalen des arktischen Gebietes. Fauna Arctica. Jena. Bd. 6, Lief. 1: 1-20.  
——— 1932-3 Acanthocephala. Bronn's Klassen und Ordnungen des Tierreichs. Leipzig. Bd. 4, Abt. 2, Buch 2.  
VAN CLEAVE, H. J. 1916 *Filicollis botulus* n. sp., with notes on the characteristics of the genus. Trans. Amer. Micros. Soc. 35 (2): 131-134.  
——— 1939 A new species of the acanthocephalan genus *Polymorphus*, and notes on the status of the name *Profilicollis*. J. Parasitol. 25 (2): 129-131.  
WITENBERG, G. 1932 Acanthocephalen-Studien II. Ueber das System der Acanthocephalen. Boll. di Zoologia (Naples) 3 (5): 253-266.

# STUDIES ON THE LIFE HISTORY OF *BRACHYLECITHUM AMERICANUM* N. SP., A LIVER FLUKE OF PASSERINE BIRDS<sup>1</sup>

J. FRED DENTON<sup>2</sup>

University of Georgia School of Medicine, Augusta, Georgia

Between 1938 and 1941 attempts were made to determine experimentally the life cycles of the common dicrocoeliid trematodes occurring in hosts in the vicinity of Houston, Texas. The results of the first of these studies were given in a recent paper (Denton, 1944) in which the morphology and development of the larval stages of *Eurytrema procyonis* Denton, 1942, were described. The present paper, the second of the series, gives in detail the results previously reported in an abstract (Denton, 1941) of experimental studies on the life history of *Brachylecithum americanum* n. sp. In this investigation, which was conducted simultaneously with the one on *E. procyonis*, the same techniques of handling and infecting snails and the same methods of study were employed (for methods, see Denton, 1944). For experimental infections, laboratory-bred snails were used throughout.

## BIOLOGY AND LIFE HISTORY

The adult *B. americanum* is a common parasite in the biliary ducts of the livers of certain birds of the families ICTERIDAE and CORVIDAE. In a series of birds from Georgia, Tennessee, and Texas, 65 per cent of the meadowlarks, 50 per cent of the mesquite grackles, 18 per cent of the bronzed grackles, and 22 per cent of the crows examined harbored this trematode. One of 12 blue jays examined contained immature forms which were assigned to this species. Individual birds have been found infected with from a few to more than 100 specimens of this worm.

Since adults of *B. americanum* could not be induced to discharge their mature eggs in either physiological saline or tap water, and since the avian hosts of this worm were found harboring other dicrocoeliids whose eggs are indistinguishable from those of this species, only eggs teased from the uteri of worms were used in the feeding experiments. Worms from meadowlarks collected in the vicinity of Houston, Texas, supplied the eggs employed in this study. The eggs, after incubation at 38–39° C for 48 hours, were mixed with a little cornmeal and fed to the following terrestrial snails: *Polygyra texasiana* (Moricand), *Stenotrema fraterna aliciae* (Pilsbry), *Mesodon thyroidus* (Say), *Praticollega berlandieriana* (Moricand), *Anguispira alternata* (Say), *Bulimulus alternatus mariae* (Albers), and *Deroceras agrestis* (Linnaeus). The eggs hatched in all seven species of snails but development proceeded only in *P. texasiana* and *P. berlandieriana*. As well as could be determined, all mature eggs had hatched within three hours, the hatching occurring in the upper small intestine of the host. The miracidia apparently penetrated the intestinal wall in this region and passed to the middle digestive gland where they continued their development.

Received for publication, September 29, 1944.

<sup>1</sup> A contribution from the Department of Biology, The Rice Institute, Houston, Texas.

<sup>2</sup> The writer expresses his appreciation to Professor Asa C. Chandler under whose direction this investigation was done, to Dr. Paul Bartsch of the U. S. National Museum for identification of the snail hosts, and to Dr. H. J. Reinhard of the Texas Agricultural Experiment Station for the identification of certain insects.

In the first experiment, *B. americanum* eggs were fed to 13 adult *P. texasiana*, of which six developed heavy infections, one failed to become infected, one examined on the 35th day after exposure may have been infected, although no sporocysts were detected, and five died and decomposed before examination. The experiment was repeated using 20 adult snails, of which 13 developed infections and seven died and disintegrated. In the first attempt to infect *P. berlandieriana*, eggs were fed to 20 very young snails, 15 of which died and decomposed before examination; the remaining 5 developed heavy infections. On repetition of this experiment, using 15 adult snails, 11 developed heavy infections, two died and were not examined, and two examined on the 35th day after exposure may have contained infections that were not detected.

In the laboratory the rates of development in the two molluscan hosts were almost identical. Mother sporocysts in both hosts were first detected with the dissecting microscope on the 50th day of infection. Three snails examined at this time contained 5, 8, and 10 mother sporocysts respectively. With the exception of one organism situated just beneath the epithelium, all the sporocysts were located in the haemocoelic space deep between the lobes of the middle digestive gland. The mothers already contained recognizable daughter sporocysts. Counts of the daughter embryos developing in different mothers ranged from 50 to 70. All of the daughter sporocysts were at approximately the same stage of development.

The mother sporocysts, when again studied on the 64th day, had increased considerably in size. In five snails examined at this time, the mothers were greatly distended by the enclosed daughter sporocysts. Each comprised a mass of daughters which almost completely filled the body cavity, leaving very little matrix. The daughters were at the migrating stage and seemed ready to emerge from the parents.

Some time between the 64th and 70th day of infection the mother sporocysts ruptured and liberated their offspring in the haemocoelic space of the hosts. In a snail examined on the 70th day of infection and in all snails examined thereafter the mother sporocysts had ruptured. Apparently, the rupturing of the mother sporocyst results from continuous stretching caused by the growth of the enclosed daughters. After rupturing, the remains of the mother sporocysts seemed to be absorbed, since no evidence of them was found in snails examined on later dates. There was no evidence that daughter sporocysts might sometimes complete their development without emerging from the parent form.

When the migrating daughter sporocysts were placed in 0.5 per cent saline, they were very active and capable of extending to three times their contracted length. The anterior "neck" was especially mobile and showed lateral movements suggestive of those of nematodes.

After liberation from the parent form, the daughter sporocysts became dispersed in the haemocoelic space throughout the body of the snail. In a snail dissected on the 70th day of infection, sporocysts were most numerous in the digestive gland but were present also in connective tissue along the intestine, in the albumen gland, in the wall of the kidney, and in the respiratory portion of the mantle. In all snails examined thereafter, sporocysts were found throughout the soft tissues of the body. Some were present even in the dorsal part of the foot. The preferred site for development seemed to be the branches of the pulmonary vein, but because of the heavy experimental infections (the snails contained 3,000 to 4,000 sporocysts) it was impossible for all of them to crowd into this location.

By the 100th day of infection the daughter sporocysts contained some fully formed cercariae along with germ balls in various stages of development. However, it was not until the 106th day that cercariae were expelled in the culture dishes from infected *P. texasiana*, and the 109th day from *P. berlandieriana*. The cercariae escaped from the daughter sporocysts through the cervical birth canal and passed into the mantle cavity where they collected in compact masses of from 150 to 300, held together by a slimy, viscid material secreted from their large glands. These cercarial masses were then expelled from the respiratory pore and were deposited on vegetation and other objects as the snails crawled about. Occasionally, a few cercariae were expelled mixed with the feces.

In the laboratory, individual snails expelled cercarial masses almost every day. Usually the masses were expelled during the night though some were expelled late in the afternoon. Just what factors controlled the escape of the cercariae into the mantle cavity, and then their expulsion in slimy masses were not determined. It is probable that in nature such factors as sunshine, and dry and rainy weather, which greatly affect the snails, in turn exert an influence on the cercariae.

Masses of cercariae were observed at different times and under various conditions after being expelled in the culture dishes and on leaves of several plants. Under no conditions were either group or individual cysts formed. When placed in water or 0.5 per cent saline the cercarial masses immediately dissolved in the liquid. In the culture dishes they dried up in a short time unless the atmosphere was kept saturated. However, cercarial masses deposited on transpiring leaves remained moist until the death of the cercariae.

The number of cercariae which developed in individual snails was enormous. Two heavily infected *P. texasiana* expelled from one to five cercarial masses daily for over a month and were still expelling them at a slower rate after three months. Each of these snails is conservatively estimated to have produced well over 20,000 cercariae.

Freshly expelled cercariae slowly extended and contracted within the viscid mass. When the mass was dissolved in water or 0.5 per cent saline, the cercariae immediately became very active. The body was extended until it was long and narrow, then rapidly lashed about, bending laterally and dorso-ventrally. At the same time the tail was slowly extended, then suddenly snapped ventrally in a tight coil (Fig. 5), the cercariae apparently trying actively to bore into or cling to something. After about 30 minutes of this intense activity, however, they were almost spent. They became sluggish and inched slowly over the bottom of the container, and in 6 to 8 hours were dead. Cercariae left undisturbed in masses deposited on leaves of plants remained alive for 24 to 36 hours. After the cercariae died, the mass liquified and disintegrated.

Although *Dicrocoelium dendriticum* (Rudolphi, 1819), the only member of the subfamily DICROCOELIINAE Looss, 1899, for which the complete life cycle has been worked out, has been shown to be capable of infecting the definitive host without utilizing a second intermediate host, there is some reason to believe that this is not a uniform condition in the subfamily, and may, indeed, be exceptional. In this form the cercariae become enclosed in slime balls which are more or less hardened peripherally, forming resistant, cyst-like structures.

In the closely related genus *Eurytrema*, according to observations made by the writer (Denton, 1944) the cercariae do not escape from the daughter sporocysts



at all, but are contained in "endocysts" inside the parent, the body wall of the latter forming an additional protective covering. Whether or not these forms require second intermediate hosts has not been determined, but it is possible that they do, since attempts to infect the definitive hosts (crow and blue jay) of *Eurytrema* sp. by feeding sporocysts that had recently escaped from a snail were unsuccessful (unpublished data).

In some of the other dicrocoeliids of birds, including *B. americanum* here under consideration, there is reason to believe that a second intermediate host might be required. Several unsuccessful attempts were made to infect definitive hosts (blue jays) with *Lutztrema* sp. both by feeding expelled cercariae and by feeding infected snails (unpublished work). It was also observed that all cercariae of this species fed to birds had disappeared from the mouth and oesophagus, and were digested in the stomach, within 30 minutes after feeding. Furthermore, it seemed improbable that the cercariae of *Brachylecithum* and *Lutztrema*, unprotected by cyst-like structures of any kind, were destined to be picked up and swallowed by their definitive hosts, which are predominantly insect-eating birds.

On the basis of these considerations, attempts were made to find a second intermediate host. Careful dissections of a number of snails with mature infections showed that encystment does not occur in snails with primary infections. Furthermore exposure of uninfected snails of several species to snails expelling cercariae showed that no transfer of cercariae between individuals with subsequent encystment occurs. It was evident that the encystment host, if one is utilized, must be sought for among the other invertebrates eaten by birds.

Earthworms and many types of arthropods which conceivably might serve as encystment hosts were tested. These invertebrates were exposed to infection in various ways. Some were placed for various lengths of time in the cultures with snails expelling cercariae; some were fed cercariae on lettuce and other plant leaves; and some were exposed by smearing cercariae on the surface of their bodies. These attempts were without success until 10 dicrocoeliid metacercariae, morphologically indistinguishable from those of *B. americanum*, were recovered from a 12-spotted cucumber beetle, *Diabrotica duodecimpunctata*, which had been exposed to the infected snails for two days. Three of the metacercariae from this beetle were enclosed in clear hyaline cysts, while the others were free in the fat bodies. The fact that several of the nonencysted metacercariae seemed to be partly chitinized suggested a previous natural infection. Whether the living metacercariae were acquired from the experimental infection or naturally, there could be no doubt of their belonging to this species or one very closely related to it.

Following this clue to the probable group of insects involved, seven adults and six larvae of the chrysomelid beetle, *Gastroidia cyanca*, were exposed to cercariae on dock (*Rumex* sp.) leaves given them for food. When examined from one to five days later, the larvae contained from one to six nonencysted metacercariae in their abdominal cavities. These were the same in every respect as the *B. americanum* cercariae to which they had been exposed, except for the lack of the tails. The adult beetles contained no cercariae. Whether the cercariae entered the larvae by mouth or by some other route was not determined. While it is possible that the beetle larvae had acquired natural infections prior to collection, the chance of all six larvae having acquired such infections just prior to the experimental exposure,



which would have to be postulated since the metacercariae were still free in the body cavity, seems too remote to be given very serious consideration.

The above observations, though not conclusive, suggest that chrysomelid beetles serve as the natural second intermediate host of this worm, and that birds become infected from eating beetle larvae containing encysted metacercariae or adult beetles which acquired metacercariae during their larval stages. This hypothesis could not be tested at the time because of insufficient material, and further work on the problem by the writer has not since been feasible. The observations made are recorded in the hope that although incomplete, they may serve as a guide to further work on this group of flukes.

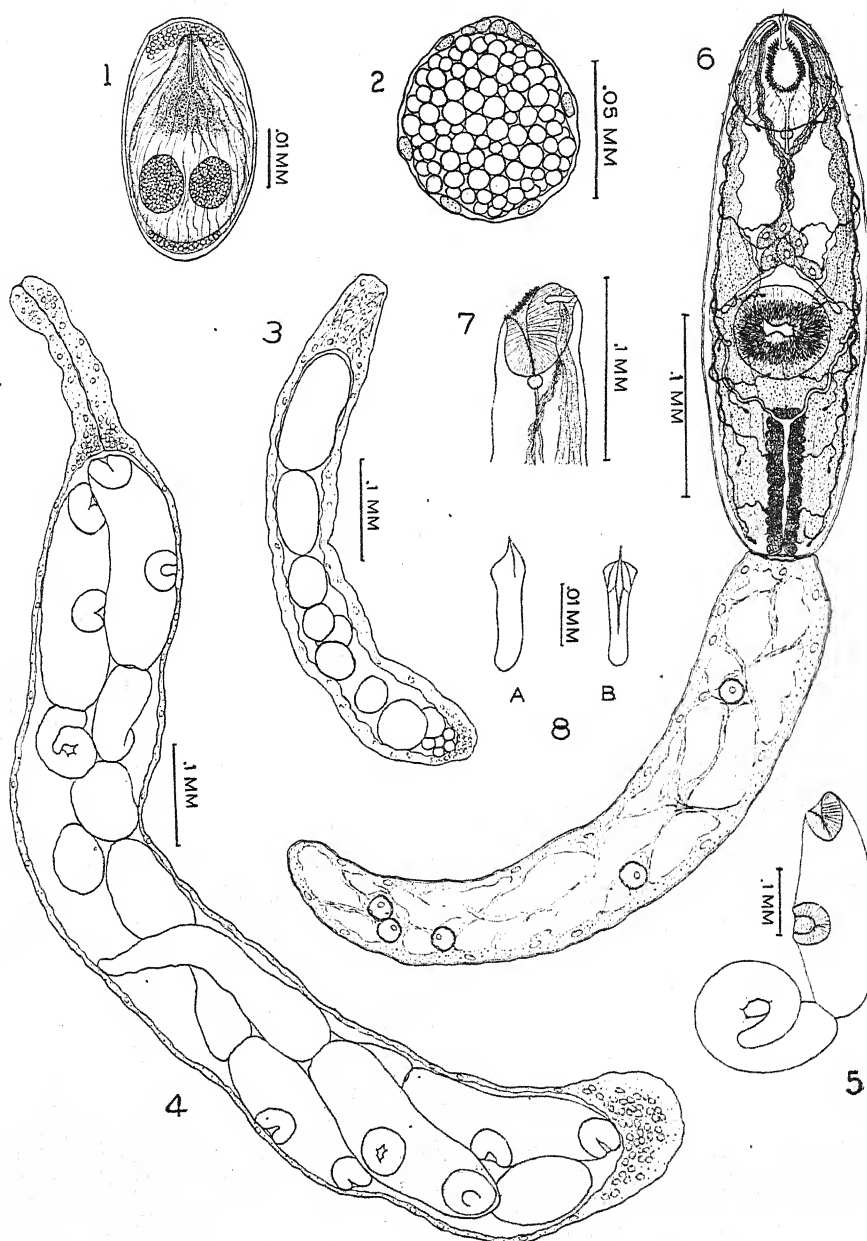
#### DESCRIPTION OF DEVELOPMENTAL STAGES

*The egg and miracidium* (Fig. 1).—Mature egg elongate oval in shape, dark brown in color, distinctly operculate, measuring  $33\text{--}46\ \mu$  in length by  $17\text{--}20\ \mu$  in breadth. Shell thick and uniform in structure except for slightly thickened posterior end. Fully developed miracidium faintly visible through opaque shell. Many refractile droplets present between shell and larva, particularly at anterior and posterior ends. Body of miracidium pear-shaped, with smaller anterior end directed toward operculum. Cilia of epidermis most numerous and longest at anterior end. Stylet slender, protruding slightly from anterior end and extending posteriorly about one-fourth of body length. Primitive gut opening through minute pore in anterior tip of body. Gut becoming broader and sac-like internally and filling most of anterior half of body. Two large oval oppositely situated vesicles containing highly refractile granules occupy posterior half of body. No epidermal plates, flame cells, or penetration glands detected in unhatched miracidia.

*The mother sporocyst*.—Mother sporocysts, observed on the 50th day of infection, were pearly white in contrast to the tan digestive gland of the host. A sporocyst developing on the surface of the gland appeared as an oval slightly raised papule, while others situated between the lobes of the gland were of various irregular shapes, their shape being determined by the space in which they were developing. Superficially, because of the bulging of the enclosed daughter embryos, the mother sporocyst has the appearance of a bunch of grapes. The thin delicate wall of the sporocyst fuses with the surrounding host tissue making it impossible to dissect it out whole. The matrix of the sporocyst is composed of irregularly shaped cells. The 50 to 70 daughter sporocysts developing in the meshes are dispersed rather uniformly through the matrix. All of the daughter embryos, in the same mother, are approximately in the same stage of development.

*The immature daughter sporocysts*.—The daughter embryos contained in the mothers on the 50th day were oval or slightly elongate structures (Fig. 2), capable of slight movement of the anterior end when freed in 0.5 per cent saline. They varied from 0.065 to 0.245 mm in length and from 0.057 to 0.126 mm in width. The wall of the smaller embryos consisted of a thin membrane composed of flattened cells, while the contents consisted of a mass of tightly packed separate germ cells. The larger embryos contained several clusters of germ cells, interpreted as being cercarial embryos at an early stage of development.

When next studied on the 64th day of infection, the daughter sporocysts, though still contained in the mothers, were at the migrating stage of development (Fig. 3).



## EXPLANATION OF FIGURES

Drawings made with the aid of a camera lucida.

- FIG. 1. Egg with mature miracidium, showing stylet and large refractive bodies.  
 FIG. 2. Very young embryo separated from mass of mother sporocyst 51 days after infection.  
 FIG. 3. Daughter sporocyst in the migrating stage 64 days after infection.  
 FIG. 4. Mature daughter sporocyst, with developing and mature cercariae.  
 FIG. 5. Lateral view of cercaria showing the ventral coiling of the tail.  
 FIG. 6. Ventral view of cercaria showing morphological details. The large and small penetration glands and their ducts are somewhat diagrammatic. Details of excretory system added freehand.  
 FIG. 7. Lateral view of anterior end of cercaria, showing position of stylet and openings of gland ducts.  
 FIG. 8. Stylet of cercaria. A—lateral view, B—dorso-ventral view.

They were elongated, cylindrical structures measuring from 0.25 to 0.60 mm in length and from 0.06 to 0.09 mm in diameter at the widest portion. The anterior end was attenuated into a solid "neck." The body cavity, which was fully formed, contained from 6 to 15 fairly large cercarial embryos and a group of smaller germinal elements. The group of small germ balls and cells, usually located at the posterior end of the cavity, is believed to be a germ mass from which additional cercarial embryos are formed. The largest cercarial embryos contained at this time measured 0.045–0.095 mm long by 0.025–0.042 mm wide. As yet, they showed no differentiation of cercarial characters.

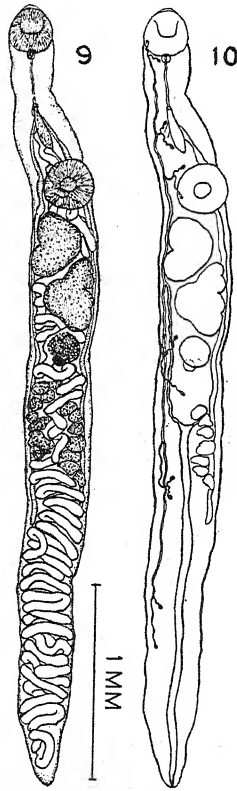


FIG. 9. Adult. Ventral view, unflattened.

FIG. 10. Adult. Ventral view, showing details of excretory system.

*The mature daughter sporocyst* (Fig. 4).—Living sporocysts glistening white in appearance, slightly motile. Total length 0.8–1.2 mm. Sporocyst divided into anterior "neck" and body proper. "Neck" narrower than body, measuring 0.10–0.20 mm in length by 0.04–0.06 mm in diameter. Birth canal passing through center of "neck" visible only when cercariae are escaping or after preservation. Diameter of body 0.125–0.175 mm at widest portion. Body wall 3–5  $\mu$  thick and composed of a single layer of flattened cells except at posterior end where it is much thicker. Body cavity containing from 8 to 15 mature cercariae and several embryos of various sizes.

*The cercaria* (Figs. 5, 6, 7).—Body relatively transparent, elongate oval in shape and flattened slightly dorso-ventrally. Ten heat-killed specimens measure 0.285–

0.360 mm long by 0.066–0.087 mm wide in region of acetabulum. Tail large, round, tapering gradually from base to tip, and measuring 0.285–0.450 mm long by 0.066–0.108 mm wide near base. It is composed of five long radially arranged giant cells supported by numerous small cells. In living cercariae the content of the giant cells is fluid and the nuclei shift their positions readily. Cuticle aspinose and smooth except for an undetermined number of sensory papillae visible along lateral margins of body of living specimens. Oral sucker directed anteriorly, muscular, measuring 0.045–0.056 mm long by 0.042–0.045 mm wide, and bearing several irregular rows of fine spines around its subterminal opening. Stylet (Fig. 8) small, measuring 22.5–24  $\mu$  in length, 5–5.5  $\mu$  in width and 6.5–7  $\mu$  in depth, and lying in small pocket in dorsal wall of sucker. Acetabulum situated just posterior to middle of body, measuring 0.05–0.06 mm in diameter, and bearing several concentric rows of fine spines on its exposed surface. Pharynx globular, measuring 8.5–10  $\mu$  long by 9–13  $\mu$  wide. Esophagus long and slender, passing posteriorly to bifurcate into two short ceca just in front of acetabulum. Six pairs of large unicellular glands lying just beneath the lateral and dorsal surfaces occupy most of the posterior half of the body. The glands become attenuated and grouped into two bundles of six, one on each side of the body, anterior to acetabulum. Each group of glands passes through lateral walls of oral sucker and opens by a common duct into the stylet pocket. A group of six small penetration glands lies in midline just anterior to acetabulum. The ducts of these glands, after tangling together, separate into two groups of three ducts each. Each group of ducts passes laterally and anteriorly through oral sucker to stylet pocket just medial to large gland ducts. Excretory pore terminal in groove between body and tail. Excretory bladder simple, tubular, I-shaped, enclosed in large compact cells, and extending anteriorly almost to acetabulum to receive a common collecting tubule from each side of the body. Each common collecting tubule passes antero-laterally, then gives rise to an anterior and posterior main collecting tubule, each of which gives off three accessory tubules that branch into two capillaries. Each capillary tubule terminates in a flame cell establishing a flame cell formula of  $2 [(2+2+2) + (2+2+2)]$ . Germinal primordia visible in stained specimens as a compact group of deeply stained cells lying just posterior to acetabulum.

*The metacercaria.*—Three encysted metacercariae, believed to be those of *B. americanum* were obtained from the abdominal cavity of a cucumber beetle. The cysts were exceedingly delicate, and changes in osmotic relations caused them to rupture soon after they were dissected out in 0.5 per cent saline. In contrast to the yellowish-green beetle tissue, the cysts were a grayish white color. The outer portion of the cyst consisted of a fairly thick layer of host tissue while the inner portion consisted of a thin layer of transparent noncellular material. Except for the absence of a tail, the encysted cercariae were no different from nonencysted forms. They showed no increase in body size or development of the germinal primordia and still retained their stylets.

*The adult worm* (Figs. 9, 10).—Body thin, semi-transparent, elongated, with almost parallel sides, rounded anterior and tapering posterior ends, 2.11–5.67 mm long by 0.158–0.564 mm wide at acetabulum. Cuticle thin, aspinose, with small retractile sensory papillae, visible on margin of preacetabular region of body. Oral sucker weakly muscular, 0.10–0.34 mm in diameter, subterminal to a short lip-like

projection. Acetabulum weakly muscular, protrusile, 0.10–0.32 mm in diameter, situated within anterior fourth of body. Pharynx small, 0.030–0.080 mm in diameter. Esophagus narrow and very thin-walled, straight to slightly wavy, 0.103–0.386 mm long, bifurcating  $\frac{1}{2}$ – $\frac{2}{3}$  the distance from oral sucker to acetabulum. Ceca of medium width, slightly wavy, passing lateral to genital organs, dorsal to vitellaria, and terminating just beyond the last vitelline follicles. Excretory pore terminal, excretory vesicle tubular, narrow, extending anteriorly to near anterior limits of vitellaria to receive a common collecting tubule from each side. Each of the common collecting tubules passes laterally and anteriorly to about posterior level of anterior testis to divide into an anterior and posterior main collecting tubule. Both of the main collecting tubules on each side of body give rise to three short accessory tubules, each of which branches into two capillaries. Each capillary tubule terminates in a single flame cell, thus establishing a 2 [(2+2+2) + (2+2+2)] type of flame cell pattern. Genital pore median at intestinal bifurcation. Testes large, distinctly lobed, approximately equal in size, 0.120–0.572 mm long by 0.090–0.400 mm wide. Anterior testis usually separated from acetabulum by a single uterine loop, posterior testis obliquely situated close behind anterior so that their zones partly overlap. Cirrus sac elongate, pyriform, 0.116–0.303 mm long by 0.050–0.103 mm wide, containing a much convoluted seminal vesicle and eversible cirrus. Ovary much smaller than testes, round to transversely oval, 0.070–0.255 mm in transverse diameter, situated to one side of body immediately behind posterior testis. Seminal receptacle small, globular, located dorsal to caudal margin of ovary. Mehlis' gland diffuse, situated medially just posterior to seminal receptacle. Laurer's canal not observed. Vitellaria each composed of 8–15 large oval follicles, located laterally just posterior to ovary. Uterus much convoluted, filling most of postovarial portion of body, then ascending by one of the three following courses to genital pore; 1, passing to either right or left of ovary, between ovary and posterior testis, between testes, then anteriorly by a wavy course; 2, passing to either right or left of both ovary and posterior testis, between testes, then anteriorly by a wavy course; 3, passing to either right or left of all three genital glands, then anteriorly by a wavy course. Mature ova few, dark brown, 33–46  $\mu$  long by 17–25  $\mu$  wide.

*Hosts:* *Cassidix mexicanus prosopidicola* (type), *Quiscalus versicolor*, *Sturnella magna*, *Corvus brachyrhynchos* and *Cyanocitta cristata*.

*Habitat:* Liver.

*Localities:* Eagle Lake, Tex. (type), Houston, Tex., Reelfoot Lake, Tenn., Athens, Ga., and Washington, Ga., U. S. A.

*Specimens:* U. S. Nat. Mus. Helm. Coll. Nos. 36752 (type), 36753 and 36754 (paratypes).

#### DISCUSSION

The genus *Brachylecithum* was established by Strom (1940) for a large group of closely related species formerly assigned to the genus *Lyperosomum* Looss, 1899. At present, the genus contains approximately 35 species and subspecies. As a group these are very delicate worms which show considerable morphological variation with different methods of handling. They also exhibit considerable variation as a result of host, habitat, age, and hereditary factors. Because of the variation shown by them, and because essential taxonomic characters are lacking for many



species which were inadequately described or described from poor material, it is impossible to determine with certainty whether or not *B. americanum* is a new species. However, since it is the first *Brachylecithum* to be reported from North American birds, it seems advisable to describe it as new. It differs from most species of the genus in having a relatively small ovary and large lobed testes situated so that their zones overlap. From the apparently related *B. papabejani* (Skrjabin and Udinzew, 1930), *B. americanum* differs in having smaller eggs and the acetabulum equal in size to the oral sucker rather than much smaller.

The morphology and development of the larval stages has been described for two species belonging to two genera of DICROCOELIINAE; *Dicrocoelium dendriticum* (Rudolphi, 1819) by Vogel (1929), Mattes (1933, 1936) and Neuhaus (1936, 1938) and *Eurytrema procyonis* Denton 1942, by Denton (1944). Of these two species, the larval forms of *D. dendriticum* show a closer affinity to those of *B. americanum* than do those of *E. procyonis*. The mother sporocysts are identical in structure and in the production of a single generation of daughter sporocysts, all of which mature simultaneously. The daughter sporocysts of both are elongated tubular structures with a distinct wall composed of a single layer of flattened cells. They both possess a birth canal. The daughter sporocysts of *D. dendriticum* are much larger than those of *B. americanum*.

The cercariae of the two species are similar with respect to general shape of the body and tail; a small stylet is present in both, large and small penetration glands occupy similar positions, and the excretory systems are identical in pattern. The cercaria of *D. dendriticum* is approximately twice the size of that of *B. americanum* and has twice as many large posterior penetration glands. The number of small penetration glands is the same for both species.

The cercariae of both *D. dendriticum* and *B. americanum* are deposited on vegetation in the same manner by the snail hosts. The cercariae of *D. dendriticum*, however, form group cysts on vegetation and are infective directly for the final hosts, while, according to the evidence here presented, those of *B. americanum* may utilize an encystment host.

#### SUMMARY

Ova of *Brachylecithum americanum* n. sp., when fed to *Polygyra texasiana* (Moricand) and *Practicollera berlandieriana* (Moricand), develop into irregularly-shaped mature mother sporocysts in 64 days. Within the matrix of the mother sporocysts a single generation of 50 to 70 daughter sporocysts develops simultaneously. The daughter sporocysts escape by rupture of the mother between the 64th and 70th day of infection and migrate to the walls of the mantle cavity and adjoining tissues to continue their development. Cercariae, mature after the 105th day of infection, escape through the birth canal of the sporocysts and collect in the mantle cavity of the host. Masses of 150 to 300 cercariae are expelled from the respiratory pore and are deposited on vegetation by the snails. The cercariae possess a long tail, a stylet, both large and small penetration glands, and a flame cell formula of  $2 [(2+2+2) + (2+2+2)]$ . Evidence is presented that the cercariae enter larvae of chrysomelid beetles which then serve as second intermediate hosts, and as a means of transfer to the definitive host. The adult worm is a common parasite of birds of the families CORVIDAE and ICTERIDAE.

The larval stages of *B. americanum* are compared with those of *Dicrocoelium dendriticum* (Rudolphi, 1819), which they resemble very closely.

## BIBLIOGRAPHY

- DENTON, J. F. 1941 Studies on the life history of a dicrocoeliid trematode of the genus *Lyperosomum*. J. Parasitol. 27 Suppl: 13-14.
- 1944 Studies on the life history of *Eurytrema procyonis* Denton, 1942. J. Parasitol. 30: 277-286.
- MATTES, O. 1933 Experimentelle Untersuchungen über die Zwischenwirtsfrage von *Dicrocoelium lanceatum*. Verh. deut. zool. Ges. 35: 227-231.
- 1936 Der Entwicklungsgang des Lanzettegels *Dicrocoelium lanceatum*. Z. Parasitenk. 8: 371-430.
- NEUHAUS, W. 1936 Untersuchungen über Bau und Entwicklung der Lanzettegel-Cercarie (*Cercaria vitrina*) und Klarstellung des Infektionvorganges beim Endwirt. Z. Parasitenk. 8: 431-473.
- 1938 Der Invasionweg der Lanzettegelceracarie bei der infection des Entwirts und ihre Entwicklung zum *Dicrocoelium lanceatum*. Z. Parasitenk. 10: 476-512.
- STROM, J. 1940 Notes on the classification of the Dicrocoeliinae (Trematoda). Magasin de Parasit. de l'Institut. Zool. de L'Acad. des Sciences U. S. S. R. 8: 176-188.
- VOGEL, H. 1929 Beobachtungen über *Cercaria vitrina* und deren Beziehung zum Lanzettegelproblem. Arch. Schiffs- u. Tropen-Hyg. 33: 474-489.

# AN ECOLOGICAL STUDY OF THE HELMINTH PARASITES OF AMPHIBIANS AND REPTILES OF WESTERN MASSACHUSETTS AND VICINITY

JOHN S. RANKIN, JR.

Department of Zoology, University of Connecticut, Storrs

Comparatively few species of helminth parasites have been described from New England fresh-water and terrestrial vertebrates. Practically nothing has been published concerning the ecology of such parasites. While associated with the Biology Department of Amherst College, Amherst, Mass., from 1936 to 1941, the writer carried on a survey of helminths of the more common species of vertebrates found in that region. Records were made of physical conditions of localities in which hosts were collected, position of worm in the host, condition of host, season, life cycles of parasites (when possible), etc., as well as the morphology and taxonomy of parasites found.

Elucidation of several trematode life cycles resulted from this study (Rankin, 1939, 1939a, 1944, 1944a), in which type of life cycle was correlated with as many ecological factors as possible. The present paper summarizes and discusses the data obtained from examinations of amphibians and reptiles. Those for birds and mammals will appear in a later publication. A total of 262 hosts was examined, including 13 species of amphibians and six species of reptiles. From these animals, 27 species of helminth parasites were collected: 12 trematodes, 10 nematodes, 4 cestodes, and 1 acanthocephalan.

It is a pleasure to acknowledge the assistance of former colleagues at Amherst College, particularly of Dr. O. E. Schotté and Mr. Carl Holthausen, in the collection and care of animals studied.

## MATERIALS AND METHODS

Animals were placed in cloth sacks immediately upon collection with damp moss or leaves to prevent desiccation. By this procedure they could be kept alive in a cold room (c. 4° C) for two or three weeks. At the laboratory, the smaller animals were dissected directly under the dissecting microscope, whereas the larger ones were eviscerated first and then the body contents studied under the microscope. All helminths were placed in Amphibian Ringer's Solution as they were encountered and studied alive at the end of each autopsy.

Trematodes were killed by placing them on a slide with a drop of water, covering with cover-glass, placing F.A.A. fixative at one edge, and drawing it under the cover-glass by means of filter paper placed at the opposite edge. Uniform fixation without distortion was insured by this method. Whole mounts were made from specimens stained in Borax-Carmine, Lynch's precipitated method, and mounted in balsam.

Cestodes were stretched around a glass plate and killed by painting them with F.A.A. fixative. Whole mounts were made from scolices and representative proglottids stained in dilute Delafield's hematoxylin and mounted in balsam.

Nematodes and acanthocephalans were killed by plunging them into hot 70%

---

Received for publication, February 2, 1945.

## SUMMARY OF HOSTS AND PARASITES ENCOUNTERED

Number of hosts examined, parasites found, and total per cent infection of each species of host with the species of parasite, are summarized as follows:

Host	No. examined	Parasite	Per cent infected
AMPHIBIA: CAUDATA			
<i>Ambystoma jeffersonianum</i> (Green)	1	<i>Brachycoelium salamandrae</i>	100
<i>Ambystoma maculatum</i> (Shaw) ..	13	<i>B. salamandrae</i>	84 +
<i>Desmognathus f. fuscus</i> (Raf.) ...	13	<i>B. salamandrae</i>	15 +
		<i>Oswaldocruzia pipiens</i>	23 +
<i>Eurycea b. bislineata</i> (Green) ....	9	<i>B. salamandrae</i>	100
		Spirurid cysts	11 +
<i>Plethodon cinereus</i> (Green) .....	35	<i>B. salamandrae</i>	25 +
		<i>Cosmocercoides dukae</i>	8 +
		<i>O. pipiens</i>	3
<i>Triturus v. viridescens</i> Raf. ....	138	<i>Plagitura parva</i>	5 +
		<i>P. salamandra</i>	42 +
		<i>B. salamandrae</i>	15 +
		<i>Gorgoderina attenuata</i>	29 +
		<i>Megalodiscus temperatus</i>	16 +
		Spirurid cysts	50 +
		<i>Capillaria tenua</i>	35 +
		<i>Camallanus</i> sp., larva	0.08 +
		<i>Physaloptera</i> sp., immature	0.08 +
		<i>C. dukae</i>	3 +
		<i>Bothriocephalus rarus</i>	0.08 +
		<i>Pomphorhynchus bulbocolli</i>	0.08 +
		<i>Plerocercoid larvae</i>	0.08 +
AMPHIBIA: SALIENTIA			
<i>Hyla crucifer</i> Wied. ....	6	<i>O. pipiens</i>	50
		<i>Glypthelminis quicta</i>	18 +
		<i>C. dukae</i>	18 +
		<i>Rhabdias ranae</i>	18 +
<i>Rana catesbeiana</i> Shaw .....	1	<i>G. attenuata</i>	100
		<i>Cephalogonimus americanus</i>	100
		<i>Halipecus amherstensis</i>	100
		<i>Cylindrotacnia americana</i>	100
		<i>C. dukae</i>	100
<i>Rana clamitans</i> Latreille .....	10	<i>G. attenuata</i>	60
		<i>G. quicta</i>	30
		<i>Loxozonus bicolor</i>	10
		<i>Haematotoechus medioplexus</i>	20
		Spirurid cysts	20
		<i>C. dukae</i>	40
		<i>Capillaria tenua</i>	20
		<i>O. pipiens</i>	60
<i>Rana pipiens</i> Schreber .....	3	<i>G. attenuata</i>	33 +
		<i>H. medioplexus</i>	33 +
<i>Rana palustris</i> LeConte .....	9	<i>G. attenuata</i>	22 +
		Gorgoderid cysts	22 +
		<i>H. medioplexus</i>	11 +
		<i>O. pipiens</i>	55
		<i>B. salamandrae</i>	11 +
		<i>R. ranae</i>	22 +
		<i>C. tenua</i>	11 +
<i>Rana sylvatica</i> LeConte .....	11	Gorgoderid cysts	27 +
		Cestode cysts	9 +
		<i>O. pipiens</i>	100
		<i>R. ranae</i>	45 +
		Plerocercoid larvae	9 +
<i>Bufo fowleri</i> Hinckley .....	1	<i>O. pipiens</i>	100
REPTILIA: TESTUDINATA			
<i>Clemmys insculpta</i> (LeConte) ....	1	Negative	0
REPTILIA: SERPENTES			
<i>Coluber c. constrictor</i> L. ....	1	<i>Kalicephalus a. flagellus</i>	100
		<i>Polydelphis</i> sp.	100
		<i>Capillaria</i> sp.	100
<i>Lampropeltis t. triangulum</i> (Lacepede) .....	1	Cestode cysts	100
		<i>R. ranae</i>	100
		<i>Capillaria</i> sp.	100
<i>Storeria dekayi</i> (Holbrook) .....	2	Negative	0
<i>Storeria occipito-maculata</i> (Storer)	1	<i>C. dukae</i>	100
<i>Thamnophis s. sirtalis</i> (Lin.) ....	6	<i>G. attenuata</i>	16 +
		Strigeid metacercariae	33 +
		<i>Zeugorthis aequatus</i>	16 +
		Cestode cysts	16 +
		<i>K. a. flagellus</i>	16 +
		<i>R. ranae</i>	33 +

alcohol. They were then transferred to a mixture of 70% alcohol and a few drops of glycerine. Some were mounted in balsam after gradual infiltration with Borax-Carmine stain. A few anterior ends of nematodes were mounted in glycerine jelly to present an *en face* view.

The lack of balance between the numbers of different species of hosts examined is chiefly a reflection of their relative abundance. The newt (*Triturus*) is by far the most common cold-blooded vertebrate in the aquatic habitats studied. The red-backed salamander (*Plethodon cinereus*) is very abundant in certain terrestrial areas, rare in others. The various species of frogs are about equally represented, although the record for the bullfrog (*Rana catesbeiana*) does not seem to show this. Ease of collecting is a pertinent factor. Salamanders are fairly easy to catch whereas frogs prove more difficult. The number of examinations for reptiles is regrettably low. These animals occur sporadically, except for the common striped snake (*Thamnophis s. sirtalis*).

#### ECOLOGY OF PARASITES ENCOUNTERED

##### Trematoda

##### 1. *Brachycoelium salamandrae* (Froehlich), Dujardin, 1845.

Hosts: *Ambystoma jeffersonianum*, *A. maculatum*, *Desmognathus f. fuscus*, *Eurycea b. bislineata*, *Plethodon cinereus*, *Triturus v. viridescens*, *Rana palustris*.

Position: Intestine.

The synonymy and relationships of this trematode have been reviewed by Rankin (1938). Worldwide in distribution, *B. salamandrae* is one of the commonest amphibian trematodes encountered (Brandt, 1936; Rankin, 1937), occurring in 53% of the amphibians studied. A correlation seems to exist between occurrence of this fluke and habitat of the host. In the strictly aquatic salamander, *Triturus*, only 15% infection is found. Likewise, but few worms per host are observed. On the other hand, terrestrial salamanders are heavily infected (25-100%). Whenever large numbers of flukes are present, the majority are quite small. Conversely, when but few (below 20) are present, individuals are usually much larger in size.

##### 2. *Plagitura parva* Stunkard, 1933, and *P. salamandra* Holl, 1928.

Host: *Triturus v. viridescens*.

Position: Intestine.

Stunkard (1936) considers the two species of *Plagitura* to be specifically valid, largely on size differences at maturity. In the present study, such great variation is found in size and maturity that much doubt is thrown on the validity of these two species. As in infection with *Brachycoelium*, heavy infections tend to produce many small flukes, whereas light infections are usually made up of large specimens. The same discrepancy was found earlier (Rankin, 1937), so much so, that in collecting all specimens were lumped together in one species. Studies on other genera of trematodes indicate that host species play an important role in determining adult morphological variations in flukes (Rankin, 1938, 1944, 1944a).

This trematode is found in only one species of host in the aquatic habitat.

##### 3. *Gorgoderina attenuata* Stafford, 1902.

Hosts: *Triturus v. viridescens*, *Rana catesbeiana*, *R. clamitans*, *R. pipiens*, *R. palustris*, *R. sylvatica*, *Thamnophis s. sirtalis*.

Position: Bladder.



The life cycle of this fluke, with a concomitant review of the literature was made by Rankin (1939). It was found that cystocercous cercariae, liberated into the water, were ingested by tadpoles, in which the young flukes penetrated to the coelom and encysted. The worms become adult in the bladder of the definitive host when the latter eat the tadpoles. The occurrence of metacercariae in the coelom of adult hosts, therefore, may be accounted for by the penetration of cercariae during the tadpole stage of the host. Likewise, such a life cycle determines the occurrence of this worm in aquatic hosts. Such hosts are usually susceptible to parasitism with this fluke.

4. *Glyphthalmus quieta* (Stafford, 1900) Stafford, 1905.

Hosts: *Hyla crucifer*, *Rana clamitans*.

Position: Intestine.

This parasite occurs only in aquatic hosts. Rankin (1944) showed that the metacercariae develop in the epithelium of frogs and that the worms become adult when frogs eat sloughed epithelium. The two localities in which this parasite was found, are very small, transient ponds.

5. *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932.

Host: *Triturus v. viridescens*.

Position: Bladder.

The genus *Megalodiscus* has been well reviewed by Bravo (1941) and the life cycle of *M. temperatus* by Krull and Price (1932). The occurrence of this fluke is limited primarily to two permanent ponds in Pelham, each connected by a small canal.

6. *Cephalogonimus americanus* Stafford, 1902.

Host: *Rana catesbeiana*.

Position: Intestine.

It is believed that this is an accidental infection. The locality from which the single host was collected is a very small sink-hole abounding in muskrats heavily infected with this fluke. Brandt (1936), however, has recorded it from anuran hosts.

7. *Halipegus amherstensis* Rankin, 1944.

Host: *Rana catesbeiana*.

Position: Mouth cavity and Eustachian tubes.

The life cycle and relationships of this fluke have been reviewed by Rankin (1944a). Metacercariae develop in copepods.

8. *Loxogenes bicolor* Krull, 1933.

Host: *Rana clamitans*.

Position: Cysts in bile duct.

A single case of infection was obtained with this fluke.

9. *Haematoloechus medioplexus* Stafford, 1902.

Hosts: *Rana clamitans*, *R. pipiens*, *R. palustris*.

Position: Lungs.

Krull (1931) showed that metacercariae of this species develop in aquatic insect larvae. Members of this genus are common parasites of frogs (Brandt, 1936; Harwood, 1932).

10. *Zeugorchis aequatus* Stafford, 1905.

Host: *Thamnophis s. sirtalis*.

Position: Stomach.

A single infection was encountered.

## 11. Strigeid metacercariae.

Host: *Thamnophis s. sirtalis*.

Position: Cysts on mesentery.

A single infection of this larva was found. Usually adult in fish-eating birds and mammals, the presence of this larva in a reptile is probably accidental.

## Cestoda

1. *Bothriocephalus rarus* Thomas, 1937.Host: *Triturus v. viridescens*.

Position: Intestine.

Only one infection with this tapeworm was found.

2. *Cylindrotaenia americana* Jewell, 1916.Host: *Rana catesbeiana*.

Position: Intestine.

A single infection was found.

## 3. Plerocercoid larvae.

Hosts: *Rana sylvatica*, *Triturus v. viridescens*.

Position: Intestine.

Single infections were obtained in each species of host.

## 4. Cestode cysts.

Host: *Thamnophis s. sirtalis*.

Position: Mesenteries and heart.

A single infection was found.

## Nematoda

1. *Oswaldocruzia pipiens* Walton, 1929.Hosts: *Desmognathus f. fuscus*, *Plethodon cinereus*, *Hyla crucifer*, *Rana clamitans*, *R. palustris*, *R. sylvatica*, *Bufo fowleri*.

Position: Foregut.

A widely distributed nematode (Harwood, 1932), this parasite is commonly found in aquatic hosts, rarely in terrestrial. It is the commonest nematode found in this study.

2. *Cosmoceroides dukae* (Holl, 1928) Travassos, 1931.Hosts: *Plethodon cinereus*, *Triturus v. viridescens*, *Hyla crucifer*, *Rana catesbeiana*, *R. clamitans*, *Storeria occipito-maculata*.

Position: Hindgut.

Harwood (1932) found this nematode to be one of the most widely distributed of all encountered. It ranges in both aquatic and terrestrial habitats.

3. *Capillaria tenua* Mueller, 1932.Hosts: *Triturus v. viridescens*, *Rana clamitans*, *R. palustris*.

Position: Lining of walls of foregut.

Predominant in *Triturus*, it is occasionally found in the other two hosts.4. *Capillaria* sp.Hosts: *Coluber c. constrictor*, *Lampropeltis t. triangulum*.

Position: Foregut.

Although immature, the species is very similar to *C. serpentina* Harwood, 1932.5. *Rhabdias ranae* Walton, 1929.Hosts: *Hyla crucifer*, *Rana palustris*, *R. sylvatica*, *Lampropeltis t. triangulum*,

*Thamnophis s. sirtalis*.

Position: Lungs.

Few specimens were found in each of the hosts.

6. *Kalicephalus agkistrodontis flagellus* Harwood, 1932.

Hosts: *Coluber c. constrictor*, *Thamnophis s. sirtalis*.

Position: Intestine.

Few specimens were found in each of the hosts.

7. *Polydelphis* sp.

Host: *Coluber c. constrictor*.

Position: Intestine.

Only one infection was found.

8. *Camallanus* sp.

Host: *Triturus v. viridescens*.

Position: Intestine.

Only one immature specimen was found.

9. *Physaloptera* sp.

Host: *Triturus v. viridescens*.

Position: Intestine.

Only one immature specimen was found.

## 10. Spirurid cysts.

Hosts: *Eurycea b. bislineata*, *Triturus v. viridescens*, *Rana clamitans*.

Position: Wall of foregut and stomach.

Over 50% of the newts are infected with this larval nematode. It is clearly correlated with the aquatic habitat.

## Acanthocephala

1. *Pomphorhynchus bulbocolli* Linkins, 1919.

Host: *Triturus v. viridescens*.

Position: Midgut.

A common parasite of the sucker (*Catostomus c. commersonnii*), this worm is probably accidental in the single amphibian host.

## DISCUSSION AND CONCLUSIONS

Correlation between type of habitat and species of parasite has been observed by many investigators (Pearse, 1924, 1926; Ingles, 1936; Rankin, 1937; etc.). Some species of trematodes are limited to hosts of a fairly narrow range of habitat distribution. *Brachycoelium salamandrae* is limited primarily to hosts in terrestrial habitats. Aquatic hosts might become infected either during a terrestrial larval stage (*Triturus*) or during occasional migrations to land (*Desmognathus*). *B. salamandrae* was the only trematode found in land turtles by Rumbold (1928). Its prevalence in terrestrial hosts was observed by Rankin (1937). Although the life cycle of this fluke is not known as yet, from a study of hosts' stomach contents as well as of hosts' distribution, it would seem that terrestrial invertebrates are involved. *Plagitura* spp. are limited to hosts in aquatic habitats. Stunkard (1936) described the life cycle of *P. parva*. He showed that metacercariae develop in fresh-water snails and insect larvae. The only chance for other amphibian hosts to become infected would be during the breeding season when such animals return to water. This would account for its rare occurrence in hosts other than *Triturus* (Rankin, 1937).

Along with *Plagitura*, the following trematodes seem to be specific for the aquatic habitat: *Gorgoderina attenuata*, *Glypthelmins quieta*, *Megalodiscus temperatus*, *Cephalogonimus americanus*, *Halipegus amherstensis*, *Loxogenes bicolor*, and *Haematoloechus medioplexus*. Little (1928) found aquatic more heavily infected than land salamanders, as did Rankin (1937). Rumbold (1928) and Brandt (1936), studying turtles and salientians respectively, came to this same general conclusion.

The occurrence of tapeworms is essentially correlated with an aquatic environment. Copepods are known to serve as intermediate hosts for salamander cestodes (Thomas, 1931). These crustaceans abound in the various aquatic areas studied.

Nematodes are distributed fairly evenly throughout the habitats studied, although there is a tendency for them to be found in few host species. *Oswaldocrusia pipiens*, *Capillaria tenua*, and spirurid cysts tend to be associated with the aquatic habitat. Acanthocephala are generally associated with a host in all stages of their development (Van Cleave, 1919). *Pomphorhynchus bulbocolli*, usually found in fishes, is probably limited to the aquatic region through its life cycle.

Locality within the host and the parasite's life cycle usually determine the habitat in which the parasite is found. For example, *Glypthelmins quieta* and *Gorgoderina attenuata* develop metacercariae in aquatic intermediate hosts and are limited, therefore, to aquatic definitive hosts; etc. On the other hand, host-specificity may determine a parasite's distribution. *Megalodiscus temperatus* and *Plagitura* sp., with aquatic intermediate hosts, are found in but one species of definitive host, *Triturus*. *Gorgoderina attenuata* tends to be limited to species of *Rana*. *Cephalogonimus americanus*, *Halipegus amherstensis*, *Loxogenes bicolor*, and the cestodes are found in single host species. This indicates that some species of helminths may be limited physiologically to single hosts. Although the data indicate that many of the observed parasites are specific for single hosts, elucidation of the life cycle may show that specificity, as such, is not the only factor determining distribution. Many species of helminths are, however, found in many species of hosts. *B. salamandrae* is found in seven different host species, *G. attenuata* in seven, *O. pipiens* in seven, and *C. dukae* in six. By far the majority of species are found in amphibian hosts. Habitat, life cycle, and host-specificity are, therefore, closely interrelated. A knowledge of all factors involved is necessary to determine the limiting one.

Age and size of, and seasonal variation within, a habitat may influence the extent of parasitism present. Small, old and permanent areas with no outflow tend to build up an infection once started. Such conditions may be more conducive to maintaining a high degree of parasitism than a larger, more fluid habitat with constant change within it. In Whately Glen Pond, *Triturus* is infected with seven species of helminths; in Leverett Pond with eight; in Ive's Ice Pond with five. On the other hand, the terrestrial habitats show hosts with few species of parasites. Likewise, accidental and multiple infections should be more common in small permanent habitats. *Triturus* is commonly found infected with six or more species of helminths, 13 different species collected in this study. *Rana* spp. usually harbor five or more species of flukes. Conversely, in the terrestrial habitat, *Ambystoma* sp. yielded but one species of helminth; *Plethodon*, one or two. Accidental infection with *Pomphorhynchus* and *Cephalogonimus* is found in small isolated ponds.

When large infections of a single species are found, the worms are usually small,

though mature. When few worms are present, however, they tend to be correspondingly much larger. Crowding may account for this size variation (Rankin, 1937). The fewer the parasites, the more room and food for each individual. The validity of specific identification may be influenced by this condition, particularly with respect to *Plagitura parva* and *P. salamandra*. Stunkard (1936) believes these two trematodes to be distinct species. Difficulty has been experienced in the present study in defining these two species. It is believed that this controversy warrants a brief consideration. The writer (Rankin, 1938, 1944, 1944a) has found that many so-called specifically valid characters are too variable to be of taxonomic value. Other investigators have come to this same general conclusion (Chandler, 1923; Venard, 1938). In hermaphroditic animals, with little or no chance for cross-fertilization, there may be built up over a period of time an isogenic stock, in which succeeding generations would be morphologically alike. If individuals of various phenotypes are obtained, then the differences should be environmental, not genetic. For example, if the metacercariae resulting from a single miracidium of an isogenic stock are fed to several species of definitive hosts, and many variations are obtained in the adult worms, these phenotypic expressions are probably due to the varied environments. The many recognized species of a given genus, therefore, may be but environmental variations of the same species.

As far as can be determined the distribution data recorded here are new.

#### SUMMARY

A taxonomic and ecological survey has been made of helminth parasites of amphibians and reptiles in western Massachusetts and vicinity. Two hundred and sixty-two hosts were examined. Twenty-seven species of helminths were collected: 12 trematodes, 10 nematodes, 4 cestodes and 1 acanthocephalan. Correlation of the data on the helminths with that on the habitats of the hosts was attempted. Aquatic habitats seem to lead to infection with more species and numbers of parasites, more chance of accidental and multiple infections, than do terrestrial habitats. Distribution of helminths depends on many factors, including habitat, life cycle of host and parasite, and host-specificity. Such variation is found in taxonomic characters of some flukes, as in *Plagitura* species, that doubt is raised as to their validity. A possible explanation of such conditions is offered in that many variations in an isogenic stock may be due to environmental rather than genetic factors. The distribution records for the helminths found are new.

#### LITERATURE CITED

- BRANDT, B. B. 1936 Parasites of certain North Carolina salientia. *Ecolog. Monogr.* 6: 492-532.
- BRAVO, M. H. 1941 Revision de los generos *Diplodiscus* Diesing, 1836, y *Megalodiscus* Chandler, 1923 (Trematoda: Paramphistomoidea). I and II. *Anal. Instit. Biol., Mexico*, 12: 127-146; 643-661.
- HARWOOD, P. D. 1932 The helminths parasitic in the amphibia and reptilia of Houston, Texas, and vicinity. *Proc. U. S. Nat. Mus.*, No. 2940, 81: 1-71.
- INGLES, L. G. 1936 Worm parasites of California Amphibia. *Trans. Amer. Micr. Soc.* 55: 73-92.
- KRULL, W. H. 1931 Life history studies on two frog lung flukes, *Pneumonoeces medioplexus* and *Pneumobites parviplexus*. *Trans. Amer. Micr.* 50: 215-277.
- AND PRICE, H. F. 1932 Studies on the life history of *Diplodiscus temperatus* Stafford from the frog. *Occ. Pap. Mus. Zool., Univ. Mich.* No. 237: 1-38.
- LITTLE, M. E. 1928 The parasites of salamanders. Unpublished thesis, Duke University, 21.



- PEARSE, A. S. 1924 Observations on parasitic worms from Wisconsin fishes. Trans. Wis. Acad. Sci., Arts and Letters 21: 147-160.
- 1926 The ecology of parasites. Ecology 7: 113-119.
- RANKIN, J. S., JR. 1937. An ecological study of parasites of some North Carolina salamanders. Ecolog. Monogr. 7: 169-269.
- 1938 Studies on the trematode genus *Brachycoelium* Duj. I. Variation in specific characters with reference to the validity of the described species. Trans. Amer. Micr. Soc. 57: 358-375.
- 1939 The life cycle of the frog bladder fluke, *Gorgoderina attenuata* Stafford, 1902 (Trematoda: Gorgoderidae). Amer. Midl. Nat. 21: 476-488.
- 1939a Ecological studies on larval trematodes from western Massachusetts. J. Parasitol. 25: 309-328.
- 1944 A review of the trematode genus *Glyphelminis* Stafford, 1905, with an account of the life cycle of *G. quieta* (Stafford, 1900) Stafford, 1905. Trans. Amer. Micr. Soc. 63: 30-43.
- 1944a A review of the trematode genus *Halipecus* Looss, 1899, with an account of the life history of *H. amherstensis* n. sp. Trans. Amer. Micr. Soc. 63: 149-164.
- RUMBOLD, D. W. 1928 The ecology of the helminth parasites of Testudinata. Unpublished thesis, Duke University, 77.
- STUNKARD, H. W. 1936 The morphology and life history of *Plagitura parva* Stunkard, 1933 (Trematoda). J. Parasitol. 22: 354-374.
- THOMAS, L. J. 1931 Notes on the life history of *Ophiotaenia saphena* from *Rana clamitans* Latr. J. Parasitol. 17: 187-195.
- VAN CLEAVE, H. J. 1919 Acanthocephala from fishes of Douglas Lake, Michigan. Occ. Pap. Mus. Zool., Univ. Mich. No. 72: 1-12.
- VENARD, C. E. 1938 Morphology, bionomics, and taxonomy of the cestode *Dipylidium caninum*. Ann. N. Y. Acad. Sci. 37: 273-328.

## RESEARCH NOTES

### A NEW LOCALITY FOR *TRYPANOSOMA CRUZI* IN ARIZONA

Kofoed and Whitaker (1936, J. Parasitol. 22: 259-263) reported Reduviid bugs infected with *Trypanosoma cruzi* from Arizona (Tucson) and added another species of insect vector, *Triatoma uhleri*, to the list of those harboring *T. cruzi* in the United States; 7 of 79 specimens of *Triatoma uhleri* were infected, whereas the *Triatoma protracta* found at Tucson were uninfected. Wood (1942, Am. J. Trop. Med. 23(3): 315-320) who did extensive collecting in 1939, 1940, and 1941 in the vicinity of the Alvarado Mine in Arizona, found 28, or 4.91%, of 570 bugs examined infected with *T. cruzi*. Among the infected bugs were *Triatoma longipes* and *T. rubida*. *T. protracta*, *Paratriatoma hirsuta*, and *T. protracta woodi* (?) were also collected but were uninfected.

During the period from June to November, 1943, due to the courtesy of Mr. W. J. Cummings, the author received 6 shipments of Reduviid bugs from the Tres de Mayo Mine, 15 miles north-east of Nogales, Arizona. Nogales is a hitherto unreported locality for *T. cruzi*, although in 1941 3 out of 7 bugs from this vicinity shipped to Dr. Dorland J. Davis, U. S. Public Health Service, were found infected with *T. cruzi*. Shipments made to Dr. Davis in 1942 contained no infected individuals.

The first shipment to the author in June, 1943, included 1 small, uninfected nymph and 20 dead *Triatoma longipes* which were too dried out to maintain an infection. In the second shipment (July) active crithidia and metacyclic forms of *Trypanosoma cruzi* were found in 1 out of the 3 *Triatoma longipes* sent. The infected individual was a large nymph which was teeming with the flagellates. The next shipment (August) included 2 live and 4 dead *T. longipes*. Of the 4 dead Reduviids, 2 were only recently dead and were found to be heavily infected. One of the two living bugs was also teeming with crithidial and metacyclic forms. In the fourth shipment (September), of 5 nymphs and 2 adult *T. longipes*, 2 nymphs and 1 adult bug were found infected. The next shipment (October) included 2 *Triatoma rubida*, one of which was infected; and in the next shipment (November) 1 out of the 2 *T. longipes* was infected. Thus of 16 *T. longipes* examined, 8, or 50%, were found infected; and of 2 *T. rubida*, 1, or 50%, was infected.

Since only a few bugs were examined, however, this high rate of infection may hold no statistical significance, although it is interesting to note that 3 out of 7 bugs (42.8%) sent to Dr. Davis from this locality in 1941 were infected.

All the specimens mentioned in this paper were collected in and around the house of Mr. W. J. Cummings. His house is a large adobe structure with white stucco walls to which many insects are attracted by lights within, or by reflected moonlight. Two hundred yards south of the camp is located the inner portal of a mine tunnel which is supported by a heavy loose-rock wall. In it is a group of wood-rat nests which have been occupied, according to Mr. Cummings, for the past 12 years. Due to this steady occupancy, Mr. Cummings thinks it probable that this is the site of the mammalian host of the *Trypanosoma cruzi* and the source of the infection. Other wood-rat nests in the vicinity are sparsely scattered among heavy rocks and cannot be opened for examination.—BETTY R. SCHUCK, Department of Zoology, University of California.

### INTESTINAL MYIASIS WITH *LUCILIA*

Infections with this genus of blowflies seem rare enough to warrant recording.

The patient, an Italian Prisoner of War aged 21, gave a history of intermittent diarrhea for six or seven months, with the passage of, "small worms," recently—several on the morning of December 1, 1943. A single living third-stage larva of *Lucilia* sp. was passed on December 2. Hexylresorcinol was given that day, after which several adult *Ascaris lumbricoides* were recovered, but no more maggots.—J. DAN WEBSTER, Second Lieutenant, Sanitary Corps, A.U.S., Utah ASF Depot, Ogden, Utah.

### THE SYRIAN HAMSTER, *CRICETUS AURATUS*, HOST OF *HYMENOLEPIS NANA*

The golden hamster, *C. auratus*, is a favorable host for many species of animal parasites and is being used increasingly in American laboratories as an experimental animal. Fecal examination of a shipment purchased recently from a commercial supply company, disclosed that several of the animals were infected with *Hymenolepis nana*. Since the cestode is a common parasite of rats and mice, its presence in the hamster is not surprising. But the worm is also a parasite of man, the eggs are numerous and very small and the contained oncospheres may develop in either the definitive or in an intermediate host. If as Chandler believes, "human infection is commonly acquired from eggs derived from rodents," laboratory workers and animal house attendants should be aware of possible contaminative infection.—HORACE W. STUNKARD, New York University, University Heights, New York.

AMERICAN SOCIETY OF PARASITOLOGISTS  
THIRTY-FOURTH COUNCIL MEETING, BALTIMORE, MARYLAND  
DECEMBER 16, 1944

The meeting of the Council of the American Society of Parasitologists was called to order by Dr. H. E. Ewing, President of the Society, at 2:00 PM, December 16, 1944, in the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland. Past-president W. W. Cort, Treasurer-elect R. M. Stabler, and the following officers and councillors were present: J. T. Culbertson, H. E. Ewing, G. F. Otto, E. W. Price, B. Schwartz, H. W. Stunkard, and W. H. Wright.

I. REPORTS OF OFFICERS

1. *Secretary (J. T. Culbertson)*: As of December 15, 1944, there were 570 persons on the membership roll of the Society of whom 508 lived within and 62 lived outside the continental United States. Of the total number on the roll, 464 were members in good standing and 106 were delinquent for one (50), two (31), or three (25) years. Of those persons in good standing, 423 lived within and 41 lived outside the continental United States. Sixty-six new members were elected during the year, of whom 61 were domestic and 5 foreign.

The death of one member occurred since the last Council meeting: Dr. H. L. Van Volkenberg, Professor of Parasitology, Agricultural and Mechanical College of Texas, College Station, Texas, died on October 13, 1944.

The secretary's report was accepted as read, and placed on file.

2. *Treasurer (G. F. Otto)*: The Treasurer's report was read by G. F. Otto. A summary of this report, prepared by Dr. Otto for publication, follows:

TREASURER'S REPORT FOR THE FINAL YEAR 1944  
(Dec. 6, 1943 to Dec. 1, 1944)

*Receipts*

Balance on hand Dec. 6, 1943 .....	\$2957.16
Collected during current year:	
348 Member dues applying 1944 .....	\$1383.50
17 Member dues applying 1945 .....	74.50
2½ Member dues applying after 1945 .....	10.37
31 Member dues arrearages .....	123.50
1 Member contribution .....	10.00
	<hr/>
	\$1601.87
3 Subscriptions to Vol. 29 .....	13.50
432 Subscriptions to Vol. 30 .....	1965.43
148 Subscriptions to Vol. 31 .....	673.80
7 Subscriptions beyond Vol. 31 .....	32.00
	<hr/>
	2684.73
Back volumes, numbers & portraits .....	449.06
Advertisements .....	119.92
Author's charges .....	85.80
Twenty-five volume index .....	53.00
Interest on savings account .....	12.52
Miscellaneous .....	15.30
	<hr/>
	735.60
	<hr/>
	5022.20
	<hr/>
	\$7979.36

*Debits*

Printing and handling Journal	
Volume 29 .....	\$ 598.53
Volume 30 .....	2372.63
	<hr/>
	\$2971.16

Expenses in office of Chr. Ed. Comm. ....	143.16	
Expenses in office of Secretary .....	253.12	
Expenses in office of Treasurer .....	493.66	
		<hr/>
	889.94	
Miscellaneous .....	33.05	
		<hr/>
	\$3894.15	
Balance on hand, Dec. 1, 1944 .....	4085.21	
		<hr/>
		\$7979.36

Respectfully Submitted  
and Certified Correct  
signed G. F. OTTO, *Treas.*

Audited and found correct  
ROBERT M. STABLER, *Chr.*  
W. W. CORT

Dr. Otto suggested that, since the Society has been operating at a profit, use of surplus should be considered, possibly for expanding the Journal or for establishing an endowment fund to pay dues of older members. Dr. Stunkard advised that surplus be kept liquid to provide for post-war emergencies. The Treasurer's report was accepted, subject to audit, and placed on file.

Dr. Otto moved that Council authorize the Secretary to turn the funds of the Society over to the Treasurer-elect, R. M. Stabler. Motion carried and the necessary documents were properly executed.

## II. REPORTS OF COMMITTEES

1. *Custodian of Princeton Secretarial Fund (N. R. Stoll)*: No report has been received since that submitted in September, 1944, and published in minutes of the Thirty-Third Council Meeting.

2. *Chairman of the Editorial Committee (H. W. Stunkard)*: The Journal itself represents the chief part of the Editorial Committee's report. In 1944, the Journal will consist of almost 400 pages made up of 83 manuscripts. At present, articles appear approximately four months after being accepted for publication. An August supplement to the Journal was published which included titles and abstracts of 40 papers submitted for the general program in Cleveland as well as 7 titles and 4 abstracts for a symposium on "Parasitology in Relation to the War." Although the general program had to be cancelled almost at the last moment, the symposium was held.

3. *Auditing Committee (R. M. Stabler, Chairman, and W. W. Cort)*: The Auditing Committee approved the annual report of the Treasurer to December 1, 1944, and the report of the Custodian of the Princeton Secretarial Fund to September 7, 1944. Upon motion, the Committee's report was accepted and placed on file.

## III. REPORTS OF REPRESENTATIVES

1. *Representatives to Council of A.A.A.S.*: No report was received from the representatives to the Council of A.A.A.S.

2. *Representatives to Council of U.A.B.S.*: No report was received since that submitted at the Cleveland meeting.

## IV. NEW BUSINESS

1. *Election of New Members*: Council elected to membership in the Society Major Hildrus A. Poindexter, M.C., A.U.S., on leave from Howard University, Medical School, Washington, D.C.

2. *Management of Journal*: Dr. Stunkard pointed out that but few Manuscripts were submitted for publication in the past year but expected a considerably larger number soon after the war's end. He mentioned that certain agencies had urged the journal to include reviews on foreign papers, especially those in Latin American journals, but that no action had yet been taken since the publication of such reviews was contrary to Journal policy.

Dr. Otto moved that By-Law of the Society under "Management of the Journal of Parasitology" (No. 2) be changed to read: "The Editorial Committee shall be assisted by an Editorial Board consisting of twelve members appointed by the Council for a four-year period in such a way that three members will retire and three new members shall be elected each year. These shall be elected on the basis of attainment, interest in the Society, geographical location, and representation of the various fields of the science." The motion was seconded and carried.

3. *Place for the Meeting in 1945*: It was moved that the place for a general meeting in 1945 not be selected at this time. Motion carried.

4. *Appointments*: President H. E. Ewing appointed the following persons to be representatives of the Society or members of Society committees:

a. Committee on nomenclature: E. W. Price (Chairman), B. G. Chitwood, and A. McIntosh. (Note below appointment to Committee on nomenclature of N. R. Stoll, ex officio.)

b. Committee on common names of parasites: P. D. Harwood (Chairman) and D. B. McMullen.

c. Committee on terminology of strains of avian malaria: C. G. Huff (Chairman), G. H. Boyd, and R. D. Manwell.

d. Representatives to Council of Union of American Biological Societies: A. O. Foster and A. C. Walton.

e. Representatives to Council of American Association for the Advancement of Science: J. E. Ackert, and H. J. Van Cleave.

Dr. Wright moved that Dr. N. R. Stoll be appointed an ex-officio member of the Committee of Nomenclature, in view of his membership on the International Commission of Zoological Nomenclature. Motion carried.

It was moved and seconded that the President appoint a Committee to select a new Secretary for 1945, if the present incumbent found it impossible to continue in office. Motion carried.

The meeting was adjourned at 4:50 PM.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary*.



# The Journal of Parasitology

Volume 31

JUNE, 1945

Number 3

## A FLUID MEDIUM FOR THE ENCYSTATION OF *ENDAMOEBIA HISTOLYTICA* UNDER REDUCED ATMOSPHERIC PRESSURE\*

LUCILE K. ZUCKERMAN AND HENRY E. MELENEY

Department of Preventive Medicine, College of Medicine, New York University

In cultivating *Endamoeba histolytica* in Erlenmeyer flasks for the purpose of obtaining large crops of cysts for experimental purposes we found, over a period of years, that there were periods of weeks or months when very poor crops of cysts were obtained even though good multiplication of the amoebae occurred. The media employed for encystation was basically the same as that described by Frye and Meleney (1935) for the cultivation of *E. histolytica* in Erlenmeyer flasks, although modifications were employed from time to time. A solid egg-Ringer base was overlaid with horse serum-Ringer or a solution of liver extract or Ringer's solution or normal saline, and an appropriate amount of rice flour was added. The inoculum was always the sediment from several tubes of cultures of *E. histolytica* without rice.

In addition to the irregularity of cyst production in such flasks, particles of coagulated egg, which were carried over in the washing procedure when the cysts were removed from the flasks, interfered with the separation of the cysts from the accompanying bacteria and rice. Various attempts were made to develop a completely fluid medium which would produce good crops of cysts regularly in flasks. The present paper describes a medium which has proved satisfactory.

### PRE-ENCYSTATION MEDIUM

The strains of *E. histolytica* from which cysts are to be obtained are kept in tube cultures containing slants of Cleveland and Collier's Difco *Endamoeba* medium stiffened by raising the agar content to 2% as recommended by Kessel et al (1944). The slants are overlaid with inactivated horse serum-Ringer 1-10. This medium is better than egg-Ringer slants because no particles of the solid slant break off and are carried over into the encystation medium.

### ENCYSTATION MEDIUM

The stock medium is composed of Cerophyl<sup>1</sup> extracted in our CPR medium. The CPR medium consists of cholesterol, one part per million, and Difco proteose peptone 0.5% in Ringer's solution. Cerophyl is suspended in this medium in a

Received for publication, January 27, 1945.

\* Aided by grants from the John and Mary R. Markle Foundation and from Eli Lilly and Company. The guidance of Dr. Thomas L. Snyder in the beginning of this work is acknowledged.

<sup>1</sup> Manufactured by Cerophyl Laboratories, Inc., Kansas City, Missouri. Cerophyl is a powder containing the dry leaves of young cereal plants. It is said to be rich in carotene, ascorbic acid, riboflavin, Vitamin K, grass juice factor and chlorophyl.

concentration of 2% and the mixture is autoclaved at 15 pounds pressure for 20 minutes. After cooling it is filtered through filter paper. The filtrate is a slightly cloudy greenish-blue liquid which cannot be clarified by further passing through filter paper. Seitz filtration is not employed because it was found to decrease the nutritive quality of the medium. The medium is reautoclaved and stored. A few hours before use it is dispensed in 50-ml amounts in 250-ml Erlenmeyer flasks, and is again autoclaved. Adjustment of the hydrogen-ion concentration is not necessary. Just before inoculation 5 ml of inactivated horse serum and 0.02 gram of especially prepared rice starch are added. It has been found advantageous to use pure rice starch in the form of separate, minute granules so that as little residue as possible will remain after maximum encystation is produced. The rice starch is prepared by digesting 100 grams of Difco rice powder with 2 grams of Armour's trypsin powder in 1000 ml of distilled water; 10 ml of toluene is added and the pH is adjusted to 7.0-7.2. The mixture is incubated at 37° C for 2-3 days, the pH being adjusted again if necessary after the first 24 hours. When microscopic examination shows that the rice starch is apparently free of protein material, the mixture is filtered through six double layers of cheese cloth. The filtrate, consisting of a practically pure suspension of individual starch granules, is washed three times in distilled water and dried with acetone. One gram of this rich starch is sterilized in a 250-ml Erlenmeyer flask in a hot air oven at 150° C for 90 minutes, after which 50 ml of the sterile Cerophyl extract is added to it. After thorough shaking, 1 ml of this suspension, which contains 0.02 gram of rice starch, is added to each encystation flask. The addition to the minimum amount of rich starch required to produce maximum encystation was suggested by Kessel et al (1944) for tube cultures, and the above amount was determined by us for flasks. This was done in order to minimize the amount of rice which would have to be separated from the cysts by flotation in zinc sulphate in our attempts to produce bacteria-free cysts (Snyder and Meleney, 1941).

#### INOCULUM

The sediment of three tubes of pre-encystation cultures of *E. histolytica* without rice, showing moderate growth after 48 to 72 hours is pooled and seeded into each encystation flask, and the flask gently rotated to disperse the amoebae and rice starch.

#### EVACUATION OF AIR AND INCUBATION

After inoculation the cotton plug of the flask is replaced by a sterile one-hole rubber stopper fitted with a glass tube and rubber tube with a hose cock, and sealed with a small amount of sterile glycerine to keep the flask airtight. The air in the flask is then evacuated by an oil pump to a pressure of 40 mm of mercury, the flask being gently rotated during this process. It is then incubated at 37° C for about 70 hours when it is opened and examined for maturity of the cysts. This is about the time of maximum production of cysts. If many of the cysts are immature at this time the flask is incubated aerobically for 3-4 hours and reexamined.

#### RESULTS

By this method of cultivation we have obtained excellent crops of cysts for over a year. In over 100 flasks the average number of cysts from

has been close to 4 million. The encysted cultures can be stored in the flasks at refrigerator temperature under either normal or reduced atmospheric pressure for at least two weeks without apparent injury to the cysts. After the cysts are washed, however, we have found it necessary to complete our manipulations and transfer into new media on the same day in order to obtain excystation.

This encystation medium will not support the multiplication of *E. histolytica* in flasks under aerobic conditions, although under the reduced atmospheric pressure a very abundant multiplication is obtained. We have not arrived at a satisfactory explanation of this difference. One might expect that more profuse bacterial multiplication under aerobic incubation would provide the necessary anaerobiosis for the amoebae. It is possible, however, that the medium does not contain sufficient nutrient to produce adequate multiplication of bacteria, and that the medium absorbs enough oxygen from its broad surface in the flask to prevent the development of satisfactory anaerobic conditions for the amoebae. These observations emphasize further the anaerobic character of *E. histolytica*.

#### SUMMARY

A fluid medium is described for the production of consistently large crops of cysts of *E. histolytica* in Erlenmeyer flasks. The medium consists of Cerophyl extracted in a solution of peptone and cholesterol in Ringer's solution with the addition of horse serum and rice starch. The cultures must be incubated at reduced atmospheric pressure in order to produce multiplication of the amoebae preliminary to cyst formation.

#### REFERENCES

- FRYE, W. W. AND MELENEY, H. E. 1935 The cultivation of *Endamoeba histolytica* in Erlenmeyer flasks. *Science* 81: 99-100.
- KESSEL, J. F., ALLISON, D. K., KAIME, M., QUIROS, M. AND GLOECKNER, A. 1944 The cysticidal effects of chlorine and ozone on cysts of *Endamoeba histolytica*, together with a comparative study of several encystment media. *Am. J. Trop. Med.* 24: 177-183.
- SNYDER, T. L. AND MELENEY, H. E. 1941 The excystation of *Endamoeba histolytica* in bacteriologically sterile media. *Am. J. Trop. Med.* 21: 63-73.

## THE ISOLATION OF MICROFILARIAE FROM BLOOD FOR USE AS ANTIGEN\*

MYRON B. FRANKS<sup>1</sup> AND NORMAN R. STOLL<sup>2</sup>

United States Naval Medical Research Unit Number Two and The Rockefeller Institute for  
Medical Research, Princeton, New Jersey

While it has been known for some time that extracts of adult filarids are capable of eliciting intradermal reactions and can also be used in serological tests (Taliaferro and Hoffman, 1930; Fairley, 1931), less attention has been devoted to the use of filarid embryos as test antigen.<sup>3</sup>

In the development of a technique of isolating microfilariae from the blood of dogs infected with *D. immitis*, we had two objectives: that the organisms be freed in a viable condition from the circulating blood elements and from the soluble blood substances, and that the yield of microorganisms be sufficiently high to make the process applicable to studies on human filariasis. Both of these objectives were achieved.

Our best results were obtained as follows: Sterile technic was used throughout. Blood was drawn into a flask containing 5% sodium citrate in saline until the citrate solution and the blood were in the ratio of 1:4. The citrated blood was centrifuged for 30 minutes at 2500 RPM, after which the plasma supernate was removed for separate processing. The WBC layer was drawn off and discarded. The packed red cell and microfilarial mass were brought to original blood volume with a 4:1 saline-citrate solution (normal physiological saline 4 parts and 3.8% sodium citrate one part), and the red cells were then hemolyzed with saponin. Approximately 1.0 ml of a 15% solution of saponin in physiological saline was required for each 15 ml of original blood volume. Care was used not to add saponin beyond the amount required for complete hemolysis as an excess caused a high mortality among the microfilariae. The preparation, under the microscope, at this stage showed the actively motile microfilariae and red blood cells shadows in the hemoglobin-tinged diluent.

The hemolyzed blood was centrifuged for 30 minutes at 2500 RPM after which the supernate, now practically free of larvae, was discarded. The stroma-microfilaria sediment was washed two or three times with saline-citrate solution to remove as much of the saponin as possible. The supernates were discarded. Most of the microfilariae were caught in the stroma sediment and their release required considerable effort and time. It was best accomplished by adding saline-citrate solution to the packed mixture of stroma and microfilariae, shaking vigorously to loosen the microfilariae, centrifuging at high speed for 30-60 seconds to bring down the stroma, recentrifuging the stroma-free supernate to recover its microfilarial con-

---

Received for publication, February 7, 1945.

\* The opinions and assertions contained herein are the private ones of the writers, and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large.

<sup>1</sup> Lieut. MC(S) USNR.

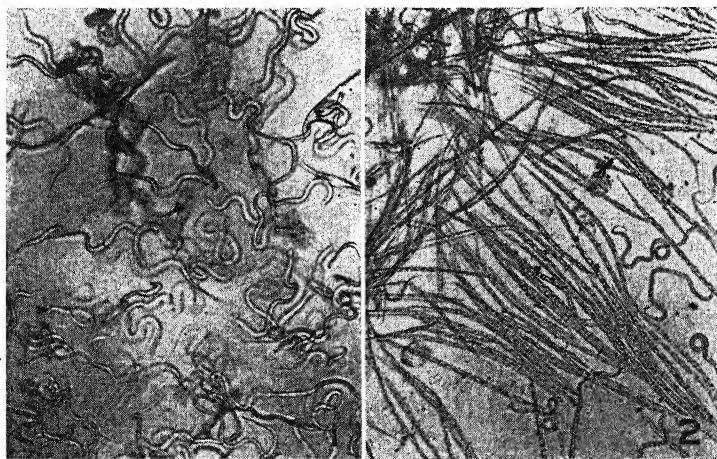
<sup>2</sup> Lieut. H(S) USNR.

<sup>3</sup> The recent report of Oliver-González and Bercovitz (1944) came to our attention after our work was terminated and this report prepared.



tent, and resuspending the stroma with more saline-citrate solution, shaking and repeating the process. A dozen washings of the stroma were sometimes needed to secure a good yield. The stage of the process requiring the shaking and the short period of centrifugation at high speed was best carried out in rubber-capped 250-ml pyrex centrifuge bottles.

The microfilariae thus isolated were in a concentration 200 or more times that of the original blood. They were then washed with physiological saline (if they were to be stored in plasma) or Tyrode solution and stored in diluted plasma, serum or Tyrode solution. In storing microfilariae so as to retain their viability, they were best kept dispersed in a shallow layer in an Erlenmeyer flask and refrigerated. Under these conditions they have routinely remained viable for two weeks or more. Fig. 1 shows a drop of living microfilariae in saline photographed with 1/200 second exposure by flash bulb.



(Photograph by J. A. Carltile)

FIG. 1. Living microfilariae in saline, after isolation from blood, photographed with 1/200-second exposure by flash bulb. (100 $\times$ .)

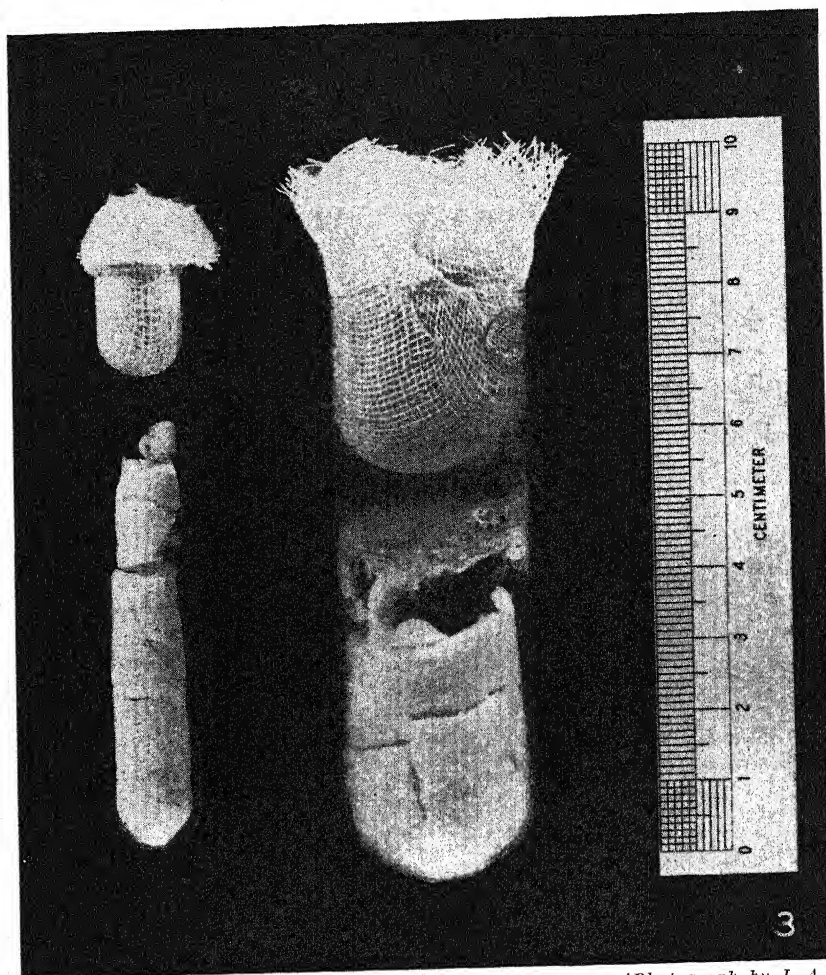
FIG. 2. The "lining up" of living microfilariae in a thin film preparation in saline photographed with 1/200 exposure by flash bulb. (100 $\times$ .)

As noted above the whole process was carried out under sterile conditions and as completely as possible in the cold. This included not only refrigeration of the various diluting solutions and sublots of blood, but immersion of flasks, tubes, centrifuge cups, etc., in iced water. To secure a yield of 80 or more per cent of the microfilariae from a liter of blood required 48–72 man hours of continuous processing. When work has been begun with the saponized hemolysate, there does not appear to be any stage at which the process may be stopped and the larvae safely stored.

To prepare the microfilariae in dry form they were further concentrated by centrifugation and washed several times with saline. As much as possible of the last saline wash was drawn off and the larvae resuspended in a small amount of distilled water, transferred to a tube of known weight and then lyophilized. Additional distilled water washes were not used in order to avoid the death of microfilariae and the leaching out of antigenic materials under hypotonic conditions. The microfilariae dried as a white, fluffy, talc-like substance (Fig. 3). Calculation from



several specimens indicated that there were 400–500 million in a gram, dry weight. They were stored at room temperature in a desiccator. Upon rewetting, the dry twisted sticks of larvae straightened and filled out to assume an almost life-like appearance (Figs. 4–5).

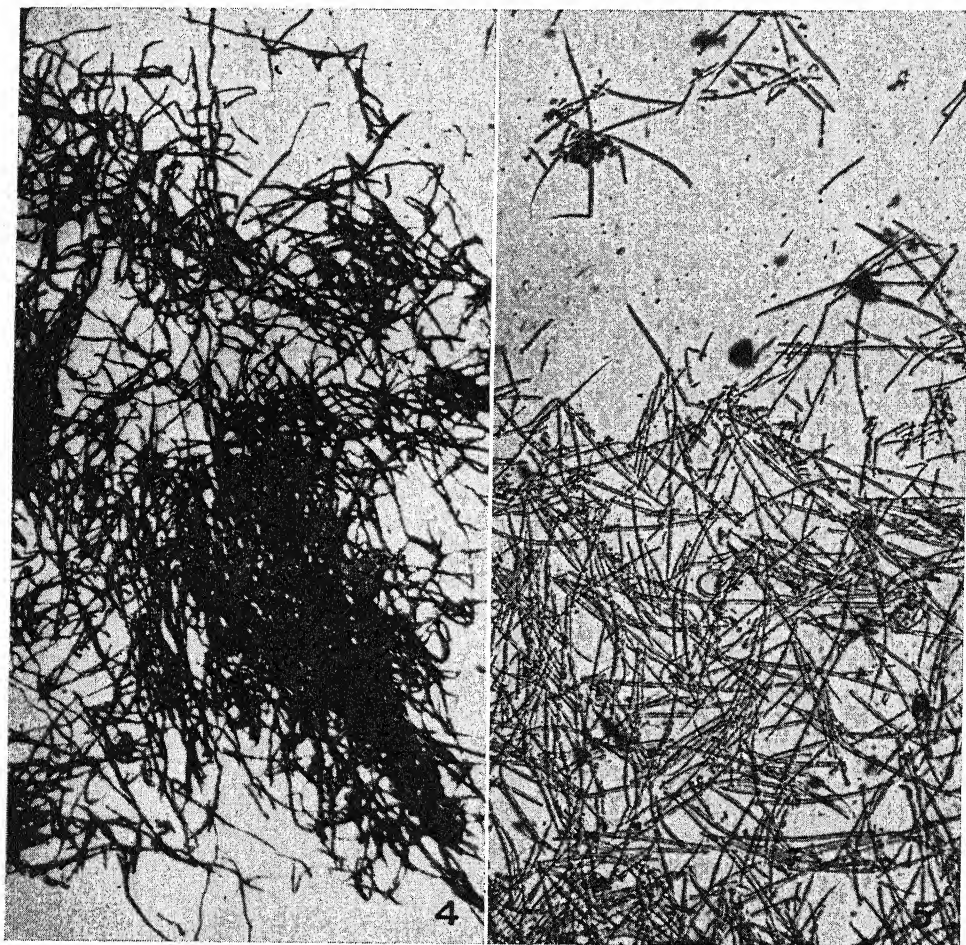


(Photograph by J. A. Carlile)

FIG. 3. Tubes of lyophilized microfilariae. Each tube shows approximately 50 million, the apparent difference in mass being due to the volume of water present when frozen.

Conditions beyond control prevented adequate serological testing of the microfilariae as a reagent but limited observations suggest their antigenic activity. The antigen was prepared on a dry weight basis with an initial dilution of 1/100 in physiological saline. The dried microfilariae were ground under sterile conditions, physiological saline was added, and the suspension kept at room temperature (26° C.) for several hours with frequent shaking, left overnight in the refrigerator and then centrifuged for 15 minutes at 3000 RPM. The supernate was used as the test antigen.

Precipitin tests were made with the sera of 20 dogs with varying degrees of microfilaremia and of 8 men known to have filariasis. All necessary control tests, negative serum, etc., were performed. The precipitin tests on the dog sera were positive at dry weight dilutions of antigen ranging from 1/1600 to 1/25,000. In many instances animals with large numbers of circulating microfilariae demonstrated low titres or gave negative reactions. The human sera reacted within the same range as the dog serum. Parallel tests set up with extracts made from dried adult



(Photograph by J. A. Carlile)

FIG. 4. Photomicrograph of wisps of dry microfilariae, photographed dry by transmitted light. (100 $\times$ .)

FIG. 5. Portion of preparation photographed in Fig. 4 upon rewetting with water.

*D. immitis* gave results similar to those with the microfilarial antigen but at a slightly lower titre. Controls were uniformly negative. Tests made with stroma contaminated antigen gave cross reaction with normal serum to a dilution of 1/1600.

In connection with the use of an extract derived from microfilariae, attention should be drawn to the difficulty of thoroughly extracting them. Besides dry grinding in a mortar, we also tried trituration in the TenBroeck grinder and after liquid air freezing. Microscopical examination of the debris following the use of these

methods showed that the microfilariae are particularly resistant to complete fragmentation. Some remained whole and apparently undamaged, others were in short cylindrical pieces with cuticle intact. Occasionally the latter would show gaping or splintered ends, but with the majority of the cells apparently undamaged. The failure of the methods used to secure the marked disintegration expected of vigorous grinding undoubtedly indicated that the extracts secured were less potent than the microfilariae are capable of furnishing.

It was noted on several occasions that when a suspension of living microfilariae was added to the serum of an infected dog, the preparation rimmed with vaseline to prevent drying and then set aside for an hour, the microfilariae gradually slowed their active wiggling and arranged themselves in orderly rows like so many matches in a box. So long as they were not allowed to dry out they maintained their viability for many hours, stayed in close approximation in a single-layer row and exhibited a gentle undulating movement. (See Fig. 2.) We thought this might be an agglutination phenomenon and checked the observation by hanging drop and thin film preparation on the serum of 26 dogs infected with *D. immitis* and 8 men known to have filariasis. In the hanging drop preparation no "lining up" of the microfilariae was noted in any of the infected serum, control serum, saline or Tyrode solution, except in those instances when the drop was not properly centered and formed a thin film at the edge of the hollow of the slide and the cover slip. As a rule the microfilariae would settle to the lowest part of the drop and maintain their activity for many hours. In the thin film preparations the "lining up" phenomenon was demonstrated not only in the immune canine and human serum but also in normal serum, physiological saline and Tyrode solution. It was, therefore, concluded that the "lining up" of the microfilariae we observed was apparently a surface tension phenomenon rather than an agglutination reaction.

## REFERENCES

- FAIRLEY, N. H. 1931 Tr. Roy. Soc. Trop. Med. and Hyg. 24: 635-648; 25: 220.  
OLIVER-GONZÁLES, J. AND BERCOVITZ, Z. T. 1944 Am. J. Trop. Med. 24: 315-316.  
TALIAFERRO, W. H. AND HOFFMAN, W. A. 1930 J. Prev. Med. 4: 261-280.

## THE STRUCTURE OF THE COMMON INTESTINAL TRICHOMONAD OF MAN

HAROLD KIRBY

Department of Zoology, University of California

In this paper an account will be given of the same trichomonad about which I published a note reporting the results of dark-field observation (Kirby, 1943). An abstract of that note by Wenyon (1944) in the Tropical Diseases Bulletin, stated that I considered the flagellate to be a species distinct from others found in the human intestine. On the contrary, I follow Wenrich (1944b) in his opinion that there is one species that is commonly found in the intestine of man; and, in fact, there are grounds for surmising that there is a possibility that all accounts of trichomonads from that situation, except that of *Tritrichomonas fecalis*, have actually dealt with the one species. A definitive description of that species has recently been written by Wenrich (1944a). This article will report certain features of the structure of the flagellate revealed by procedures different from those included in his studies and the many others that have been published.

Wenrich made his studies chiefly on specimens in fecal smears stained with Heidenhain's iron haematoxylin; and on flagellates, grown in cultures, that were dried after exposure to osmic acid fumes and stained by Giemsa's method. The two strains of trichomonads described in this paper were grown in culture in Ringer's solution plus one-eighth horse serum over an egg slant. One strain was derived from a stool specimen from a person in California in May, 1943. The other strain was sent in 1944 from the Army Medical School to the School of Tropical Medicine at the U. S. Naval Hospital, Treasure Island, California. Film preparations from these cultures were fixed in Hollande's cupric picroformal and impregnated by Bodian's protein-silver technique. The procedure was as follows: 1% aqueous protargol with 0.5 g copper wire in 10 cc (the wire coiled at the bottom of the Columbia cover glass staining dish) 12-48 hr at 37° or room temperature; washing; 1% hydroquinone in 5% sodium sulfite, 5-10 min; washing; 1% aqueous gold chloride, 2-4 min; washing; 2% aqueous oxalic acid, 2-5 min; washing; 5% aqueous sodium thiosulfate, 5-10 min; thorough washing, dehydration, and clearing. Observations have also been made by dark-field illumination.

The value in protozoological technique of silver impregnation by activated protargol, as used by Bodian (1937), was reported by Cole and Day (1940). Before their statement was published, the striking result obtainable in certain flagellates by the method was called to my attention by Dr. L. R. Cleveland.

Credit is due to Mrs. Marietta Voge for assistance in the study and for making the drawings; to Mr. J. E. Gullberg for the photographs; to Mr. Bronislaw Honigberg for maintaining the cultures; to Mr. E. N. Kozloff for making some of the preparations; to Mrs. E. N. Scott for the original specimens of the Berkeley strain used; and to Lt. A. C. Pipkin for the Army Medical School strain. The Cutter Laboratories, Berkeley, generously supplied the horse serum for the cultures, and



the Research Committee of the University of California provided financial support for the work.

#### DESCRIPTION

In suitable Bodian protein-silver preparations, with an intensity dependent on the time and temperature of impregnation, the flagella, costa, undulating membrane border, and axostyle may be shown with great distinctness. On the other hand, the nucleus varies in intensity of impregnation; often it is not blackened, but is gray (Fig. 27) or colorless; still, it is possible unmistakably to observe its size and position. The fact that the nucleus is then not heavily stained aids in observation of the small parabasal body, which lies against its membrane (Figs. 25-27), and could be differentiated only with great difficulty and uncertainty, if at all, in haematoxylin-stained preparations. The blepharoplast-complex is usually not shown, because it is obscured by the relatively large, deeply impregnating structure in the anterior portion of the body.

The general appearance of this deeply impregnating structure is not unlike that of the very large, iron-haematoxylin-stained mass shown anterior to the nucleus in some drawings of *T. hominis* (fig. 46, for example, in the paper by Bishop, 1931); but it is in striking contrast to the small blepharoplast-complex usually represented. It might be thought that the large mass is an accident of impregnation: that is what I thought at first, as it seemed possible that silver technique might be especially likely to give such a result. With further study, however, I became convinced that it is a structure definitively demonstrated, which in the ordinary iron haematoxylin preparations does not appear at all. In the silver preparations it shows in every specimen and is constant in position and shape. The structure will be referred to as the pelta. It is altogether distinct from the blepharoplast-complex.

The pelta is a membrane-like structure which has, in general, somewhat of a crescentic form (Figs. 9, 10, 16, 17). It is situated at the periphery of the body toward the right side in the most anterior part, and lies to the right of the blepharoplast-complex, which may sometimes be seen when impregnation is light as an ill-defined rounded body (Figs. 16, 17). From the point of view of the observer, in the positions in which the trichomonad is most often placed on slides, the pelta lies either over or under the blepharoplasts. Its convex anterior edge is at the extreme anterior edge of the body. The pelta is prolonged in a prominent, tapering extension in its dorsal part (Figs. 4-9). Soon after leaving the main body of the pelta the extension narrows to a filament that is comparable in thickness to the costa. In its first part it is at the periphery of the body; then it turns posteriorly and passes deeper in the cytoplasm dorsal to the nucleus and a short distance to the right of the undulating membrane and costa. There is some variation in its apparent length; in most specimens the length considerably exceeds that of the main body of the pelta. In many specimens the posterior end of the filament is posterior to the nucleus (Figs. 8, 10); in others the filament seems to be shorter (Figs. 4, 6), but it may be that it is not then impregnated for its full length. This dorsal filament extending from the pelta is present in all interphase specimens, and is relatively constant in position and form. It does not come in contact at any place with the nuclear membrane.

At the other end of the pelta (Figs. 2-4, 8, 9, 23) there is a short, narrow extension of silver-impregnating substance along the region ventral to the nucleus; this is probably on the ventral edge of the capitulum of the axostyle. It is likely



that the structure here designated as the pelta is continuous with, or part of, the capitulum. Even so, as a deeply impregnating structure the pelta has an integrity of its own. The flattened capitulum lies on the left side of the nucleus; antero-ventrally it is contiguous with the edge of the pelta, which extends on the right side of the blepharoplasts and the plane of the nucleus. The posterior part of the trunk of the axostyle—especially where it projects from the cytosome—often impregnates as deeply as does the pelta (Figs. 21, 27).

In the early prophase stage shown in Fig. 20 the pelta has left its original position, leaving the blepharoplast-complexes visible; and it has been somewhat disorganized. Although it still impregnates deeply it is evidently in process of disintegration. New peltas are probably reorganized in the course of the division process.

I formerly (Kirby, 1943) called attention to the fact that the base of the group of anterior flagella appears in dark-field illumination as a single columnar structure that shows only two edges. The independent flagellum was reported to originate in a bright, clear end entirely separate from, and a micron or so posterior to the base of the group of anterior flagella. This arrangement of the flagella appears in the same manner in the silver preparations. In addition, the pelta appears in the intervening region, and the reason for the arrangement is apparent (Figs. 9, 11, 16).

The blepharoplast-complex is situated on the left side of the pelta and the group of anterior flagella extends anteriorly from it (Figs. 16, 17). In the silver preparations the group is at the base a single columnar structure which appears solid black (Figs. 1, 3, 4, 11, 22). Farther anteriorly the group generally separates into its four component flagella (Fig. 22). The independent flagellum originates from the blepharoplast-complex and passes in an altogether different direction. It emerges under the posterior edge of the pelta, and it extends free on the right side of the body near the ventral border (Figs. 4, 7). Because of the pelta, it is mechanically impossible for the basal part of the independent flagellum to come in contact with the base of the anterior flagella. In living flagellates this flagellum is often directed dorsally on the right side, and moves anteroposteriorly in the vicinity of the undulating membrane.

The two strains that I have studied are characterized by the possession of four anterior flagella and an independent flagellum. The presence of this number of flagella in more than fifty living specimens of the Berkeley strain was reported in 1943. In suitable silver preparations of the Berkeley strain all specimens encountered in a reading of two slides were recorded. On one slide sixty-one specimens had unmistakably four anterior flagella and one independent flagellum. Twenty-nine were recorded as not countable. A few of these were degenerate, but most were not counted because the group of anterior flagella was turned back against the body, and it was not possible to be sure of the number. Also omitted were several specimens in division. In seven specimens counts were made that were not four plus one: two specimens had four anterior flagella but no independent flagellum; two had three anterior and no independent, and three had the independent but apparently only two or three anterior flagella. These seven specimens cannot in any case be interpreted as indicating genetic departure from the complement of flagella typical of fully developed specimens. Some of them were small and

could be post-division stages in which flagella had not all developed. In some the missing flagella may have been overlooked, by reason of position or staining. There might also be abnormal specimens. Some preparations, made from old cultures in which many of the flagellates were more or less moribund, showed great variability in flagella: there was loss of the structures until many had none at all—but obviously, no conclusions can be drawn from the study of degenerate specimens.

On another slide in which almost all of the flagellates were in normal condition, 125 specimens encountered in succession were recorded for flagella. A certain additional number were not recorded because, for the reasons given above, the flagella could not be counted. One hundred and eleven specimens had unmistakably four anterior flagella and an independent flagellum. The fourteen others included some that lacked the independent flagellum, some with apparently only three anterior flagella, and other departures from the normal number. In certain specimens the complement of anterior flagella included three long ones and a very short one, and in some the independent flagellum was very short. Recent beginning of outgrowth was evident in these cases. It is clear that specimens like this in no way suggest the existence of genetic variability in the typical complement of flagella in the flagellates studied.

Studies of flagella of the strain from the Army Medical School, made both by dark-field illumination and silver impregnation, gave similar results. A count of all normal specimens encountered in succession in a reading of one silver preparation gave a record of thirty-four with four anterior flagella and one independent flagellum, and four not countable.

The four anterior flagella are almost always grouped together in their basal portion. In some specimens they have retained this grouping for their whole length, and there appears only a single flagellar column (Fig. 19a). Often in the silver preparations it is possible in such a group to differentiate the ends of the four flagella—which are generally of unequal length. Usually the flagella separate at a short distance from the base. The division may be into 2, 3, or 4 parts (Fig. 19b, c, d; 17), and the thickness of each part is an indication of how many flagella compose it. Groups of two (Fig. 19b, c) or three (Fig. 17) flagella may adhere for all their length; it is evident how this adherence of flagella could mislead an observer of specimens in which they are not very clear and appear very slender. It is reasonably certain that the claim by Duboscq and Grassé (1924) that *Trichomonas trypanoides* has 1, 2, 3, or 4 flagella is based on an error of interpretation.

The independent flagellum is normally directed posteriorly, moving in an arc that generally does not carry it forward to parallel the anterior flagella. In many specimens it lies at least in part against the surface of the body; and in the majority it does not appear with the group of four anterior flagella.

The costa is black in some silver preparations, faint in others. The thickness of the costa appears in preparations where the flagella are black, to be about the same as the thickness of an anterior flagellum (Figs. 13, 24). In its posterior part, at the posterior end of the body, the costa tapers to a finer filament (Fig. 18).

The structure of the undulating membrane shows with much distinctness in suitable silver preparations. There are two filaments, one at the edge and the other closely paralleling it; they are separated by a narrow, clear space of constant width (Figs. 4-6). The filaments appear equal to one another in size. Some

specimens are impregnated so heavily that the whole structure—filaments and intervening space—appears as a black band. Near the anterior end the two filaments meet, and a single fine filament passes to the blepharoplast-complex, on the left dorsal side of the pelta. At the posterior end one of the filaments continues in the posterior flagellum, which may appear considerably stouter than the filament. The other filament terminates bluntly near the point where the posterior flagellum becomes free (Fig. 18). The filament that terminates at the end of the membrane is Wenrich's accessory filament. In agreement with Wenrich (1944b) I have observed that the accessory filament seems to be peripheral to the other one.

Often some granules are present in the region between the costa and the outer edge of the membrane; generally these are closer to the costa. Frequently, at least in places, the granules are arranged in an incomplete row. They are the paracostal granules described by Wenrich. They vary in size, and they often impregnate deeply with protargol.

The silver preparations show further features of interest at the ends of the flagella. In regard to the terminal differentiation there are two types of flagella in this flagellate: the posterior flagellum constitutes one type and the other five flagella the other type. Generally, the posterior flagellum ends in a filament (Fig. 15) that is much more slender than the rest of the flagellum and has a very variable length of up to about 5  $\mu$ . Sometimes this filament appears smooth and continuous, but more often it appears uneven and broken. The existence of this differentiation is indicated in Wenrich's account (1944a, p. 194). I am unable to say, however, whether the filament is a free axoneme or not.

The ends of the anterior flagella (Figs. 14, 21) are usually different in appearance from that of the posterior flagellum. There is no prolongation in a filament, and the end is blunt. Occasionally, the terminal part appears to be attached by a filament to the rest of the flagellum, but that is not the usual form. Sometimes the end is not distinguished from the rest in any way. Often, however, it may be seen in fixed specimens that the terminal part, up to half a micron or so in length, is bent to one side, generally slightly, but sometimes as much as a right angle. (This feature is indicated in one of the flagella in Wenrich's Fig. 4, 1944a.) This terminal structure impregnates black, and may be sharply set off in intensity of staining from the part posterior to it. It varies in length. Sometimes it appears like a black granule or a slight enlargement at the end of the flagellum. In most specimens it appears as a black rod, straight, bent, sometimes somewhat curved. It may suggest a claw or the head of a golf club. The terminal differentiation resembles in size and shape some of the bacilli that are present in very diverse size and form in this culture. But its consistent occurrence at the end of the flagella leaves no doubt that it is actually part of the flagellar structure.

Dark-field illumination of the flagellate shows the exterior structure with much distinctness. The free flagella are very substantial, solidly illuminated strands with an apparent thickness comparable to that seen in well-impregnated silver preparations. The filament at the end of the posterior flagellum can be found in many specimens, but others do not show it. With a few exceptions, no filament appears at the end of the anterior flagella, which generally terminate bluntly, in ends that are as thick as any other part. Often the terminal section, corresponding to the part that is especially deeply impregnated in protein-silver, seems especially bright

and somewhat thicker than the rest. It was sometimes seen to be bent at an angle to the rest of the flagellum, but that feature was noted much less frequently in the dark-field preparations than in the fixed specimens.

The undulating membrane margin shows in dark-field two parallel filaments, each finer than a free flagellum. This structure can be made out only in specimens in which the membrane is moving slowly, not after death of the flagellate.

The projecting part of the axostyle appears as a rod with two parallel bright edges. The projection from the cytoplasm is moderately long; and there is no taper until near the posterior end, when the rod is rather abruptly sharpened. It terminates in a point, usually without prolongation in a filament. The interior of the rod is optically empty, except for a few small granules in some instances. In the movements of the specimens observed the projecting axostyle was a rigid rod, and did not bend even passively. One specimen was dragging a dead trichomonad and a group of bacteria at the end of a rather long filament prolonged from the end of the axostyle. Even when the axostyle was jerked laterally against the resistance of this attached mass it did not bend. Eventually, the dead trichomonad became lodged between masses of debris and pulled away, leaving the axostyle of the active flagellate sharpened but without a filament.

Demonstration of the parabasal body is a particularly interesting result of the use of Bodian's protein-silver method. This structure has not been seen before in *Pentatrichomonas hominis*. Wenrich observed in some iron-haematoxylin-stained specimens a fine fibril in the position normally occupied by the parabasal body in trichomonads. He could not suggest, however, whether the structure was a parabasal fibril or a new costa. There is also the possibility that it might have been a part of the dorsal filament extending from the pelta, but the position is not the same, since Wenrich's fibril is entirely between the nucleus and the costa. The structure that I consider to be the parabasal body is deeply impregnating, small, ellipsoidal body of small size against the anterolateral surface of the nucleus (Figs. 4-12, 25-27). It is not between the costa and the median longitudinal plane of the nucleus, but lies a short distance to the right of the plane passing through those two structures. In some specimens the parabasal substance is divided into two granules (Fig. 17). Sometimes a filament (Fig. 4) can be traced from the anterior end of the parabasal until it passes under the pelta, doubtless to meet the blepharoplast. No filament was observed passing posteriorly. Protargol might not be expected to demonstrate the filament well, in the light of the reaction of the parabasal apparatus of some other TRICHOMONADIDAE to the technique. It is possible that there exists a filament comparable in its extension beyond the parabasal substance to that of *Trichomonas tenax* and *T. vaginalis*; and since Wenrich's filament is in just the position it would be expected to occupy, it may be that that is the structure.

#### DISCUSSION

From a taxonomic point of view, the most significant characteristic of the trichomonad of which an account is given in this paper is the existence of the independent posteriorly-directed flagellum. It has been observed in this and related flagellates by various authors (Kofoid and Swezy, 1923; Tanabe, 1926, 1940; Wenrich, 1944a, b; and others), and it has been known that it originates from a separate blepharoplast. Most writers have not differentiated it clearly from the group of



anterior flagella, and have written as though the five-flagellated trichomonads simply have one anterior flagellum more than the typical *Trichomonas*, as the three-flagellated species have one less. Tanabe (1940) noted that in *Pentatrichomonas felis*, which is identical with *T. hominis* according to various authors (Wenrich, 1944a), this flagellum trails immediately after emergence. Actually, the independent flagellum does not in any sense belong with the group of four anterior flagella. It is more nearly to be compared with the trailing flagellum of *Peranema* or *Eutreptia* in its position in relation to the body. It should, therefore, be recognized as a new feature of structural organization added to that of *Trichomonas*. The internal structures of the mastigont and the undulating membrane show considerable differences among trichomonads in degree of development, but I do not think that those differences are so significant for group differentiation as is this independent flagellum. It seems to me that the presence of that structure warrants generic differentiation of the intestinal trichomonad of man; and I think that the taxonomic differentiation should be recognized by assigning to the flagellate the name *Pentatrichomonas hominis* (Davaine, 1860). Wenrich, 1931, seems to have been the first to compose the name in this combination.

If, as is likely, the pelta is a development of the anterior part of the capitulum of the axostyle, a comparison may be made with the extensions of the capitulum that occur in certain DEVESCOVININAE, the highly differentiated group of TRICHOMONADIDAE in termites. The pelta is demonstrated very clearly by use of silver technique; but earlier studies, which did not include that technique, have not recorded it at all. Very little is known about the structure of flagellates as revealed by silver impregnation, and undoubtedly there is much to be learned by application of this valuable technique.

The structural differentiation of the terminal parts of the flagella is another feature whose study will be furthered by use of silver technique. Attention has been directed to structure of the flagella of many flagellates (Vlk, 1938). One type is the Peitschengeissel, so named because of the existence of a fine terminal filament. All of the flagella on *Bodo*, *Trepomonas*, and *Hexamitus* studied by Vlk were found to be of this type. It is also the type of the posterior flagellum of *Pentatrichomonas hominis*. The anterior flagella have a different structure, a substance at the ends that impregnates more deeply than the rest, and in fixed specimens may be set at an angle to the rest of the flagellum.

The very small parabasal body of *Pentatrichomonas hominis* has not hitherto been reported. Silver preparations may show the parabasal substance well, but fail to demonstrate the filament; that has been noted in various trichomonadin and devescovinin flagellates. The account of the parabasal apparatus given in this paper must, then, be regarded as not necessarily complete. Further search for a filament, which may as in *Trichomonas tenax* extend far posteriorly to the parabasal substance, must be made. It is possible that Wenrich (1944a) did see the filament, but for certainty it is necessary that both substances of the parabasal apparatus should be revealed at the same time.

The genus *Pentatrichomonas* contains intestinal flagellates of various vertebrates. Wenrich (1944a) suggested that the forms in man, monkeys, cats, dogs, and rats all belong to the one species. There is also *Pentatrichomonas macropi* Tanabe, 1926, of kangaroos; *P. eutamias* Tanabe and Okinami, 1940 of *Eutamias asiaticus*;



and perhaps the five-flagellated trichomonad from poultry, as described by Allen (1936, 1940). In *P. macropi* from "the kangaroo" the independent flagellum shown by Tanabe is comparable in length to the anterior flagella; but in *P. macropi* from four species of *Macropus* and one of *Dendrolagus* the length of the independent flagellum shown by Herman (1939) is more than twice that of the anterior flagella—a relative length that is never reached in the strains of *P. hominis* that I have studied. Because silver preparations make possible the study of additional structural detail, they would be valuable for comparison. Differences in the pelta and the parabasal body would be highly significant. Until comparisons are made with regard to those, as well as other features, it is not possible to state that all these forms are morphologically identical with one another. The flagellate which in 1931 I described under the name *Pentatrachomonoides scroa*—a trichomonad that occurs in various termites—was mistakenly listed by Kudo (1939) as *Pentatrachomonas*. Its organization is very different from that of *Pentatrachomonas hominis*. The generic name is not very suitable, since it has resulted in confusion and suggests a closer similarity than that which exists.

#### SUMMARY

In Bodian protein-silver preparations many features of the mastigont structure of an intestinal trichomonad of man can be demonstrated with much distinctness. Some of these features have not hitherto been reported. At the anterior end of the body is a flattened structure, the pelta, which lies to the right of the blepharoplast-complex and is prolonged dorsally in a filament that passes posteriorly in the cytoplasm dorsal to the nucleus. The pelta may be an extension of the capitulum of the axostyle. The margin of the undulating membrane consists of two parallel filaments, as Wenrich reported; one of these continues in the posterior flagellum, which generally terminates in a slender filament. The other flagella are typically as stout at the ends as elsewhere; and have a terminal section that impregnates especially deeply with silver, and sometimes is bent at an angle. A small, ellipsoidal parabasal body lies against the anterolateral surface of the nucleus, a short distance to the right of the plane passing through the nucleus and costa. The parabasal apparatus may also include a filament, like that in *Trichomonas tenax*, but it was not demonstrated in this material. This flagellate from man possesses a normally constant number of four anterior flagella in a group, an independent flagellum, and the undulating membrane with its posterior flagellum. The independent flagellum is directed posteriorly, arises under the posterior margin of the pelta, and is entirely separate from the anteriorly-directed group of four flagella. It is believed that this feature justifies generic distinction of this flagellate of man, as well as of similar forms in other animals. The intestinal trichomonad of man may be denominated *Pentatrachomonas hominis* (Davaine, 1860).

#### REFERENCES

- ALLEN, E. A. 1936 A *Pentatrachomonas* associated with certain cases of enterohepatitis or "blackhead" of poultry. Tr. Am. Micr. Soc. 55: 315-322.  
——— 1940 A redescription of *Trichomonas gallinarum* Martin and Robertson, 1911, from the chicken and turkey. Proc. Helm. Soc. Washington 7: 65-67.  
BISHOP, A. 1931 The morphology and method of division of *Trichomonas*. Parasitology 23: 129-156.

- BODIAN, D. 1937 The staining of paraffin sections of nervous tissues with activated protargol. The role of fixatives. *Anat. Rec.* 69: 153-162.
- COLE, R. M. AND DAY, M. F. 1940 The use of silver albumose (protargol) in protozoological technique. *J. Parasitol.* 26 Supplement: 29-30.
- DUBOSCO, O. AND GRASSÉ, P. 1924 Notes sur les Protistes parasites des Termites de France. I. *Trichomonas trypanoides* n. sp. *Compt. Rend. Soc. Biol.* 90: 547-550.
- HERMAN, C. M. 1939 *Pentatrichomonas macropi* Tanabe from kangaroos. *Zoologica* (New York) 24: 293-295.
- KIRBY, H. 1943 Observations on a trichomonad from the intestine of man. *J. Parasitol.* 29: 422-423.
- KOFOID, C. A. AND SWEZY, O. 1923 On the morphology and life history of *Pentatrichomonas ardin delteili* (Derrieu and Raynaud). *Univ. Calif. Publ. Zool.* 20: 373-390.
- KUDO, R. R. 1939 Protozoology. C. C. Thomas, Springfield.
- TANABE, M. 1926 Morphological studies on *Trichomonas*. *J. Parasitol.* 12: 120-130.
- 1940 Notes on the morphology of *Pentatrichomonas felis* from the cat. *Keizyô J. Med.* 10: 124-125.
- AND OKINAMI, M. 1940 On the parasitic protozoa of the ground squirrel, *Eutamias asiaticus* Uthensis, with special reference to *Sarcocystis eutamias* sp. nov. *Keizyô J. Med.* 10: 126-134.
- VLK, W. 1938 Über den Bau der Geissel. *Arch. Protistenk.* 90: 448-488.
- WENRICH, D. H. 1931 Morphological studies on the trichomonad flagellates of man. *Arch. Soc. Biol. Montevideo, Supp.*: 1185-1204.
- 1944a Morphology of the intestinal trichomonad flagellates in man and of similar forms in monkeys, cats, dogs, and rats. *J. Morphol.* 74: 189-211.
- 1944b Comparative morphology of the trichomonad flagellates of man. *Am. J. Trop. Med.* 24: 39-51.
- WENYON, C. M. 1944 Abstract of Kirby, H. 1943 Observations on a trichomonad from the intestine of man. *Trop. Dis. Bull.* 41: 290.

## EXPLANATION OF PLATES

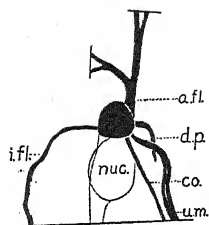
All figures are of *Pentatrichomonas hominis* (Davaine)

## Abbreviations

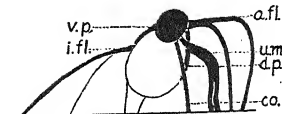
a. fl.—anterior flagella	nuc.—nucleus
ax.—axostyle	pe.—pelta
co.—costa	p. b.—parabasal body
bl.—blepharoplast-complex	p. fl.—posterior flagellum
d. p.—dorsal filament extending from pelta	u. m.—undulating membrane
i. fl.—independent flagellum	v. p.—ventral extension of pelta

## PLATE 1

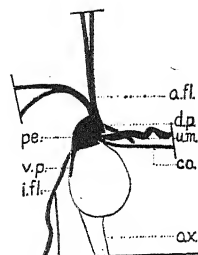
FIGS. 1-12. Camera lucida drawings of the mastigont structures of the anterior part of the body. In all specimens the anterior flagella originate in a columnar structure which later divides into four flagella. Where there appear to be two or three, actually some are united with others. The margin of the undulating membrane consists of two parallel filaments. The pelta is extended dorsally in a filament that extends deep in the cytoplasm near the costa. There is a ventral extension along the ventral edge of the capitulum of the axostyle. The parabasal body is against the nuclear membrane near the costa. In a view from the dorsal side (Fig. 12), it shows alongside the nucleus. In the other figures it lies either above or below the nucleus, but focusing indicates that it is outside the membrane. Hollande cupric microformal. Bodian protein-silver.  $\times 3500$ .



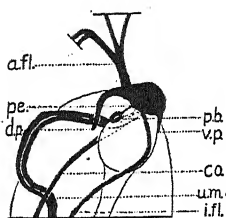
1



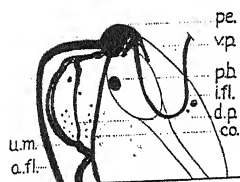
2



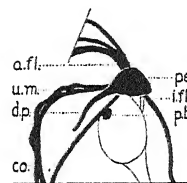
3



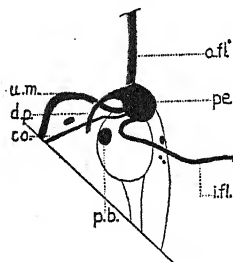
4



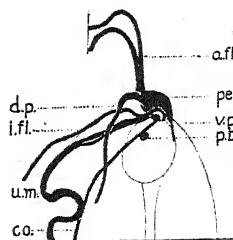
5



6



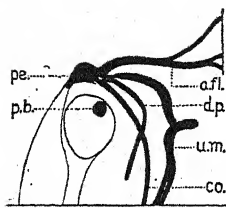
7



8



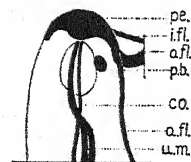
9



10



11



12

## PLATE 2

FIG. 13. *Pentatrichomonas hominis* entire. The margin of the undulating membrane consists of two parallel filaments. The dorsal filament extending from the pelta, most of which lies between the nucleus and costa in this specimen, appears also in Fig. 24, which is a photograph of the same specimen.  $\times 2400$ .

FIG. 14. Distal parts of the anterior flagella, showing the terminal differentiation that impregnates more deeply than the rest with protein-silver. The terminal differentiation shows in the photographs Figs. 21 and 22. Freehand diagrams.

FIG. 15. Terminal filaments of the posterior flagella, sometimes absent. Freehand diagrams.

FIG. 16. Portion of the mastigont of a specimen in which the pelta is not very heavily impregnated, and the blepharoplast-complex can be seen as a separate body, giving origin to the mastigont structures.  $\times 3500$ .

FIG. 17. Pelta and blepharoplast-complex can both be seen; three anterior flagella adherent in one group, one partly free. Parabasal body appears as two granules.  $\times 3500$ .

FIG. 18. Diagram of the posterior end of a mastigont, margin of the undulating membrane partly pulled away from the edge of the cytosome, which is marked by the costa. One of the marginal membrane filaments continues in the posterior flagellum; the other terminates at the end of the membrane.

FIG. 19. Diagrams to show various types of groupings of anterior flagella. The pelta is also shown. An independent flagellum is present in each specimen, but is not shown in the drawing. In *a* the four flagella are united, each has a different length, and the end of each can be seen along the column. In *b* the flagella are united in pairs. In *c* two flagella are separate, two united but of different length and the ends can be seen. In *d* the four flagella are separate except at the base.

FIG. 20. An early prophase. There are two blepharoplast-complexes, represented here somewhat diagrammatically, which give rise to sets of mastigont structures. There is a parabasal body associated with each mastigont. The original pelta is displaced, is in disorganization, and new ones have not begun to develop.  $\times 3500$ .

## PLATE 3

Photographs of *Pentatrichomonas hominis*, made by J. E. Gullberg, not retouched. Specimens fixed in Hollande's fluid, impregnated by Bodian's protein-silver technique.

FIG. 21. The independent flagellum is turned forward in a position that is not the usual one. Terminal differentiations show at the ends of the anterior flagella, and a filament on the posterior flagellum.

FIG. 22. This and the preceding figure show the origin of the four anterior flagella in one group, and the separateness from this of the independent flagellum.

FIG. 23. Shows the pelta, part of the dorsal filament that extends from it, the ventral extension of the pelta, a group of four anterior flagella, and the independent flagellum turned forward.

FIG. 24. Specimen shown also by drawing Fig. 13. Dorsal to the nucleus is the dorsal filament from the pelta.

FIG. 25. The parabasal body appears as a large rounded granule posterior to the pelta. The dorsal filament from the pelta can also be seen.

FIG. 26. Different focus on the same specimen shown in Fig. 23. Parabasal body applied anterolaterally to the surface of the nucleus.

FIG. 27. Parabasal body posterior to the pelta; near the costa a cytoplasmic granule similar in size. Two of the group of four anterior flagella are adherent until near their ends. The independent flagellum is on the ventral side of the body parallel to the posterior flagellum.

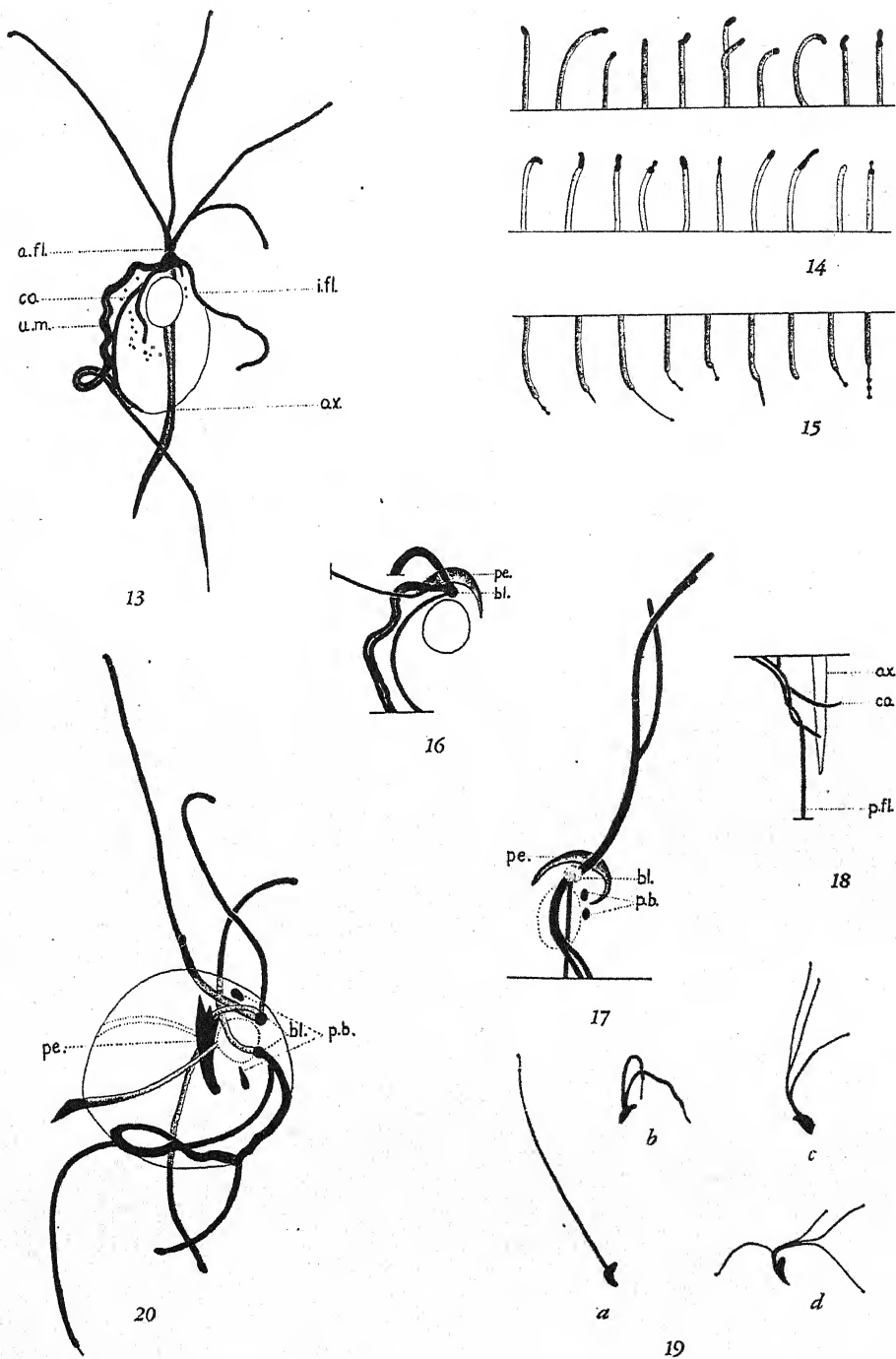


PLATE 2



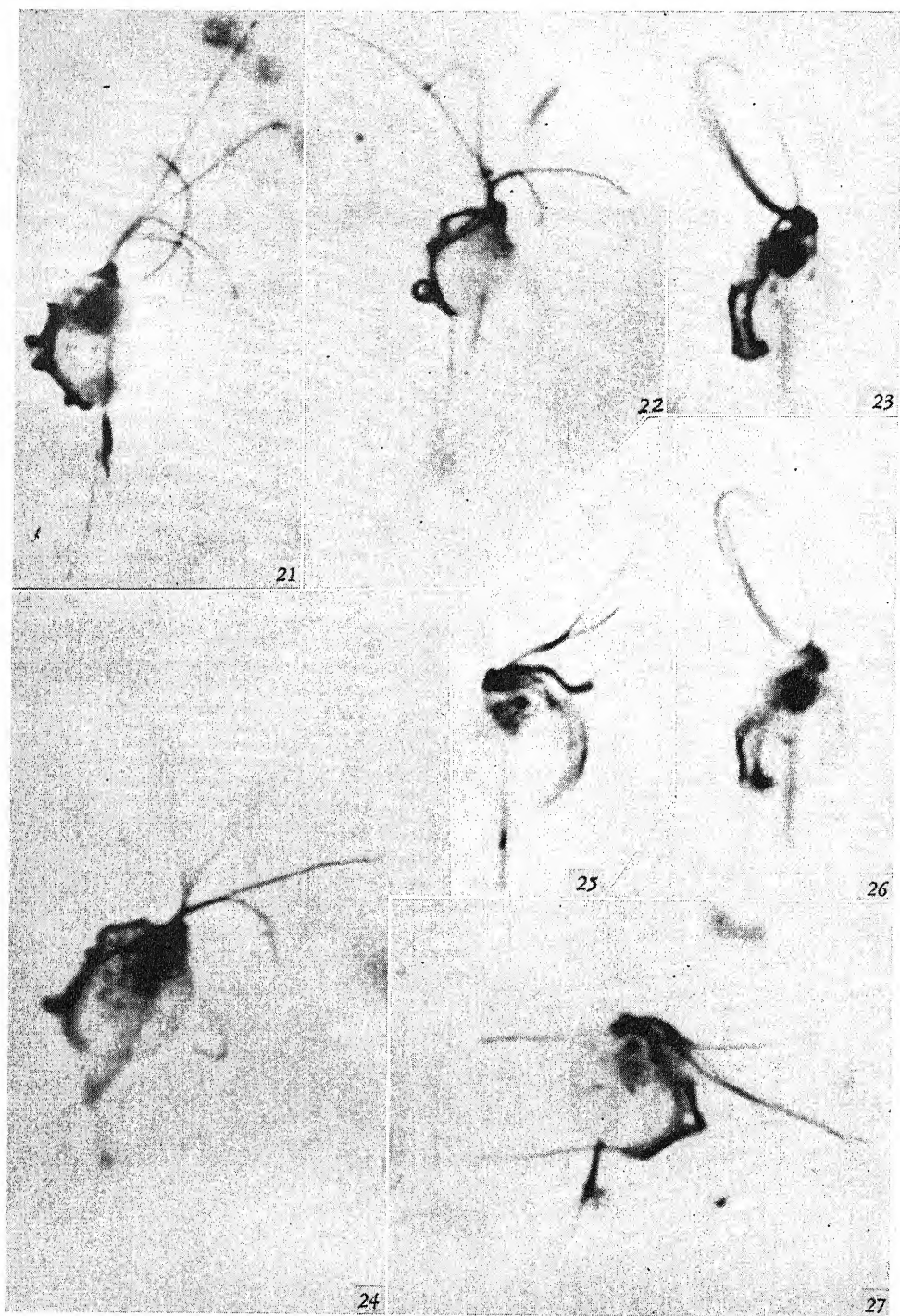


PLATE 3



## ENTAMOEBA COLI VERSUS ENDAMOEBA COLI

HAROLD KIRBY

University of California, Berkeley

In drawing up the argument for Opinion 99 of the International Commission on Zoological Nomenclature, Stiles showed a wish to reject the name *Entamoeba* in the interest of clear distinction. He wrote: "it seems obvious that unless the name *Entamoeba* is definitely suppressed both the nomenclatorial and the taxonomic status of the species which come into consideration will become even more confused." The result of his reasoning was rejection of the name, but the benefits that he hoped for have not been realized. There have been some who in following the Opinion have been influenced to take a position regarding the taxonomic status of the amoebae that is not in accord with clear distinction, because unless they took that position the necessary large nomenclatorial departure from the usage that is very widespread in the literature of medical zoology would indeed result in confusion. Retention of the two names *Endamoeba* and *Entamoeba* would permit a clear-cut taxonomic differentiation to be made at the same time that a minimum of departure from customary usage is necessitated. Therefore it seems to me that Opinion 99 has actually increased the difficulty that Stiles wished to avoid. I agree with Dobell (1938) that the Opinion in its present form should not be regarded as decisive.

The equivocal interpretation that some authors have made of Opinion 99 is illustrated by Craig's criticism (1944) of Kudo's retention of the name *Entamoeba*: "It would have been much better had he followed the ruling of the International Committee of Zoological Nomenclature and used the spelling recommended by it as preferable, i.e., 'Endamoeba.'" Kudo took the position that the species *coli* should not be put into the same genus as the species *blattae*, and his failure to follow Opinion 99 made it possible for him to choose *Entamoeba* as the generic name for *coli*. It is not a question of alternative spelling of the name of the genus: the Opinion does not constitute an approval of the spelling *Endamoeba* as against *Entamoeba*. There is no choice of orthography: *Endamoeba* is correct and has priority as the name of the genus typified by *E. blattae*; all amoebae in that genus are called *Endamoeba*, and those not in that genus cannot be called *Endamoeba*.

The Opinion was published in 1928, and so far as I know, between that time and this only two names have been used in connection with the species *coli*, *histolytica* and *gingivalis*: *Endamoeba* by those who put the three amoebae into the same genus as *blattae*, and *Entamoeba* by those who do not. The authors in the former group do not accept the generic distinction; whether or not they follow Opinion 99 does not properly enter into their adoption of *Endamoeba*. The authors in the latter group do recognize the generic distinction, and do not follow the

---

Received for publication, March 3, 1945.

<sup>1</sup> Mr. Francis Hemming has, at my request, worded a footnote for this paper as follows: As the subject discussed in the present paper relates to the possible revision of Opinion 99 of the International Commission on Zoological Nomenclature, this paper has been communicated also to the International Commission for the information of the Commissioners. In order that the International Commission may be in a position to inform itself of the views of all interested specialists, all comments on the issues raised in the present paper should be sent direct to the Secretary of the International Commission at the Commission's Publication Office, 41 Queen's Gate, London, S. W. 7.—H. K.

Opinion. If there should be a third group of authors, who recognize generic distinction among these endozoic amoebae, and accept Opinion 99, it would be necessary for them to write *Poneramoeba histolytica*, *Poneramoeba coli*, and *Poneramoeba gingivalis*.

The following chronological summary of the history of this matter sets forth the important facts that need to be considered:

1879. Leidy (1879a, p. 300) introduced the generic name *Endamoeba*, with the one species *Endamoeba blattae*, named *Amoeba blattae* by Bütschli in 1878. The same proposal was printed on December 2 in Leidy, 1879b, p. 205.

1895. Casagrandi and Barbagallo introduced the generic name *Entamoeba*, giving as the included species *Amoeba coli* (Lösch) and *Amoeba blattarum* (Bütschli). They were ignorant of Leidy's name.

1897. Casagrandi and Barbagallo (p. 163) again printed the name *Entamoeba*, giving as the included species *Entamoeba hominis* and *Entamoeba blattarum*.

1903. Schaudinn, using the generic name *Entamoeba* C. & B., divided *Amoeba coli* of Lösch into two species, *Entamoeba coli* Lösch and *Entamoeba histolytica* n. sp. He did not mention the species *blattae*, and probably was ignorant of Leidy's name.

1910. Chatton assigned various endozoic amoeba to the genus "*Entamoeba* Leidy (1879)." Among the included species were: *Entamoeba coli* (Lösch) 1875, emend. Schaudinn (1903); *E. blattae* (Bütschli) 1878; *E. ranarum* (Grassi) 1881; *E. histolytica* Schaudinn 1903. Chatton stated that the paternity of *Entamoeba* had been wrongly attributed by authors to Casagrandi and Barbagallo. He made no reference to the fact that Leidy's name was actually *Endamoeba*.

1912. Séance du 14 février, mémoire paru le 5 mars (acc. to Chatton, 1912). Chatton and Lalung-Bonnaire wrote (p. 142): "La dénomination non latine d'Entamibes, appliquée aux amibes normalement parasites du tube digestif est d'un usage commode qui la fera conserver. Mais traduite en nom générique latin, elle ne peut plus s'appliquer actuellement aux amibes du tube digestif des Vertébrés. Ce n'est pas, en effet, à ces dernières qu'elle a été appliquée en premier lieu. C'est Leidy qui a créé le genre *Entamoeba* pour l'amibe de la Blatte, et ce n'est qu'en 1897 que Casagrandi et Barbagallo l'ont appliquée aux amibes intestinales des Vertébrés." The authors considered that the amoebae of vertebrates must be put in a separate genus, for which they proposed the name *Löschia*, to contain *coli* Lösch and other species.

1912. Séance du 26 mars. Chatton reported again the generic differentiation of endozoic amoebae made in the above paper. He definitely designated *Löschia coli* Lösch, cysts 8 nuclei or more, as type of the genus *Löschia*. Remarking that protistologists had wrongly attributed the paternity of the genus *Entamoeba* to Casagrandi and Barbagallo 1897, he wrote (p. 111): "Ces derniers avaient bien appliqué le nom d'*Entamoeba* à une Entamibe humaine, mais Leidy l'avait donné des 1879 à l'*Amoeba blattae* de Bütschli." In a footnote to this statement he noted that Leidy's spelling was "*Endamoeba*," but dismissed that name as an orthographic variant.

1913. Brumpt wrote of the amoebae of man under the name "*Entamoeba* Leidy, 1879," making the same mistake for *Endamoeba* that Chatton as well as Alexeieff (1912) had previously made. In a footnote (p. 21) he wrote: "Ce même genre a



été créé de nouveau en 1897 par Casagrandi et Barbagallo pour leur *E. hominis*, synonyme de *E. coli*." That sentence has been accepted by Stiles and Boeck (1923, p. 122), Stiles and Hassall (1925, p. 8), and Stiles (1928 in Opinion 99) as a designation of the type *E. coli* (as *E. hominis*) for *Entamoeba* Casagrandi & Barbagallo. (In the third edition, 1922, Brumpt made the same error of "*Entamoeba* Leidy"; but in the next one, 1927, he used *Entamoeba* C. & B. and noted that *Endamoeba* Leidy should be reserved, in agreement with Wenyon, 1926, for the amoeba of the roach.)

1919. Dobell used *Endamoeba* Leidy, 1879, for *E. blattae* only, and *Entamoeba* Casagrandi & Barbagallo, 1895, for *E. coli*, *E. histolytica*, and *E. gingivalis*.

1923. Stiles and Boeck, in a study of the nomenclatorial status of the protozoa of man (p. 125), considered the genus *Endamoeba* Leidy, 1879, with two subgenera: *Entamoeba* Leidy, 1879 (type by monotypy *E. blattae*) and *Poneramoeba* Luehe, 1909 (type by monotypy and original designation *E. histolytica*). They stated (p. 124) that "*Entamoeba* 1895 is not available because of *Endamoeba* 1879"; evidently that is because they thought of *Entamoeba* as a homonym, or orthographic variant, because here they dealt with a separate taxonomic category (the subgenus) from *Endamoeba*. The type of *Entamoeba* 1895 is given (p. 122, 124) as *E. hominis* tsd. = *coli* and *coli* (s. *hominis*); type by subsequent designation is by Brumpt, 1913.

1925. Stiles and Hassall, in the "Key-Catalogue of the Protozoa Reported for Man," list (p. 8) *Entamoeba* C. & B., 1895, type by subsequent designation *hominis* = *coli*, as a subjective synonym of *Endamoeba* Leidy, 1879. It appears from the definition of subjective synonym by Stiles and Boeck, 1923, that it is a category providing for cases where the identity in question is not absolute, but depends on "the experience and judgment of the reviser" (p. 138). Since in the key-catalogue the types of *Entamoeba* and *Endamoeba* are given as different, although those two types are treated as members of the same genus, it is likely that the reference is to the difference of opinion about generic assignment. Otherwise *Entamoeba* would simply have been designated as a homonym; that category is dealt with in the same paper. Reference to *Entamoeba* as a synonym is, therefore, evidently on the basis that its type, *Ent. coli*, belongs in the same genus as *End. blattae*, according to Stiles and Hassall.

1928. The International Commission on Zoological Nomenclature published Opinion 99 in response to an inquiry as to whether the names *Endamoeba* and *Entamoeba* should be considered homonyms. The summary of the Opinion reads: "*Entamoeba* 1895, with *blattae* as type by subsequent (1912) designation, is absolute synonym of *Endamoeba* Leidy, 1879a, p. 300, type *blattae*, and invalidates *Entamoeba* 1895, type by subsequent (1913) designation *hominis* = *coli*." The report also contained the decision that *Entamoeba* is a homonym of ("philologically the same as") *Endamoeba*. It is presumably on that basis that the Secretary recommended that "the name *Entamoeba* 1895, either with type *hominis* = *coli* as definitely designated by Brumpt, 1913, p. 21, or with *blattae* as accepted by Chatton and Lalung (1912, 111) and as implied by Chatton (1910, 282), be definitely invalidated by *Endamoeba* Leidy, 1879a, p. 300, type *blattae*, irrespective of the point whether the type of *Entamoeba* be considered *blattae* or *coli*." (The reference to Chatton and Lalung, 1912, p. 111, is evidently a mistake for Chatton and Lalung-Bonnaire, 1912, p. 142, or for Chatton, 1912, p. 111.)



It is evident from this summary that Stiles (1928) was justified in his statement that "the case has already produced considerable confusion in literature." It is also evident, however, that this confusion need not have existed if authors had simply been attentive to the correct forms. Then *Endamoeba* Leidy would have been used for any generic concept including the species *blattae*; and *Entamoeba* C. & B. would either have been rejected, or used solely for any generic concept omitting *blattae* and including *coli*. The errors made by earlier authors should not influence us in an effort to reach a solution of the problem.

The answer to the taxonomic problem is subject to differences of opinion. Many authors follow the usage of Stiles and Boeck, 1923, in writing *Endamoeba coli*; that usage has been almost universal in American compilations in medical zoology since it was adopted in 1926 by Craig (who in 1911 used *Entamoeba* Casagrandi and Barbagallo without reference to the genus *Endamoeba* Leidy). American writers who recognize generic distinction between *blattae* and *coli* include Kudo (1939, 1944), Wenrich (*Entamoeba* used for *histolytica* and *coli* in 1940, 1944, and other papers of similar date), Cleveland (Cleveland and Sanders, 1930, and other papers), Pearse (1942), and Meglitsch (1940, in connection with a profound study of *blattae*). And there are many in various parts of the world who follow the same course; (for example, Wenyon, 1926; Dobell, 1919, 1938; Brumpt, 1936; Reichenow, 1928), so that it is not a question of individual or even minority disagreement in the question of taxonomic differentiation.

It is not my purpose in this paper to attempt to defend one position in taxonomy or attack the other. Because of the very large difference between the species *blattae* and *coli* in the nuclear structure of the trophic forms, I think that the burden of proof should rest on those who assert that the two amoebae belong in the same genus—especially when the same authors recognize as valid certain other genera of endozoic amoebae. A comprehensive analysis of the taxonomy of all amoebae, free living and endozoic, is much to be desired. Morris (1936) examined the problem so far as certain endozoic amoebae are concerned; but the result of his study is not conclusive, for it omitted from consideration certain other endozoic amoebae that would also have to have the status of subgenera of *Endamoeba*, according to his treatment. The purpose of the present paper is nomenclatorial: it is an attempt to show that the word *Entamoeba* should remain available for a genus of which *Ent. coli* is the type.

Opinion 99 declares that those of us who think that the species *coli* and similar forms do not belong in the same genus as *blattae* cannot use the name *Entamoeba* for that different genus. There are two grounds upon which that declaration is based. One of them is that *Entamoeba* is a homonym of *Endamoeba*—that the two words are not sufficiently different from one another in orthography to be usable as separate words. The other is that *Entamoeba* has the same type species as *Endamoeba*, and therefore falls as a synonym. The latter decision is the only one given in the summary of Opinion 99; it is not necessary that it should be rendered after the generic name has been dismissed as a homonym, so evidently it is intended to provide a reserve in case of doubt.

That doubt certainly exists (Dobell, 1938). Obviously we are not here concerned with whether the words have the same meaning or not, but with whether one word is the same word as the other. There is a difference between inadvertent

interchange of two names that have a status in zoological nomenclature, and the use synonymously of such words as endoplasm and entoplasm or endoderm and entoderm. There is nothing in the articles of the International Rules of Zoological Nomenclature that justifies the conclusion that *Entamoeba* must be rejected as a homonym. Certainly Chatton's statement, although cited as authoritative by Stiles, does not constitute justification; it is merely an assertion in a one line footnote, unsupported by reference to the Rules or anything else. It is only in the argument for Opinion 99 that evidence is given, but that evidence can as well be read in support of the retention of the two names as different. Jordan's report in the Opinion states that they come in the category of names of which the spelling in Latin varied to a slight extent and which the Rules of Nomenclature do not accept as different. His reference is to Article 35, in which precise differences are given by which specific names of the same origin and meaning are insufficiently distinguished. There seems to be no indication that Article 35 is intended to establish a general category allowing interpretation of other differences than those specified; and in that Article there is nothing whatever about the sort of difference that exists between the words *Endamoeba* and *Entamoeba*. Furthermore, there is evidence in Opinion 99 itself that the two words are not necessarily of the same origin, and that would exclude them from consideration under the rules given in Article 35.

Article 35 deals only with specific names, and it might seem possible that a different interpretation for generic names would be allowed. Now, however, a precise statement concerning differences in generic names has been given (Opinion 147, 1943). A generic name of the same origin and meaning as a previously published generic name is to be rejected as a homonym of the said name if it is distinguished therefrom only by certain specified differences, which are the same ones given for specific names in Article 35. Opinion 99 was not mentioned by the Commission in the rendering of Opinion 147, although it dealt with the subject that was being considered.

It is not possible to find any definite reason in the Code, or any valid evidence in Opinion 99, for rejection of *Entamoeba* as a homonym; but the recommendation in Article 36 can, as Taliaferro remarked, be evoked in support of retention of both names. These facts have already been discussed by Dobell (1938).

In Opinion 99 the consideration that is apparently regarded as the more important one, since it alone is given in the summary, is that of synonymy—that *Entamoeba* C. & B. is an absolute synonym (or objective synonym, Stiles and Boeck, 1923, p. 135) of *Endamoeba* Leidy, because the names follow their types, and the same species, *E. blattae*, is the type of each. When Stiles presented the case so as to arrive at this conclusion, he changed his approach to the matter. In 1923 he evidently regarded *Entamoeba* as a homonym, in 1925 he designated it as a subjective synonym on the basis of the taxonomic assignment of its species, but in both papers he accepted *E. hominis = coli* as type of *Entamoeba* C. & B. by subsequent designation by Brumpt, 1913. In Opinion 99, after stating that Brumpt's action was the first type designation in words, Stiles found it possible to interpret Chatton, 1912, as having designated *blattae* as type of *Entamoeba* C. & B. Stiles did not make clear the reason for this interpretation, except in that he cited Opinion 6 in support of it.

*Entamoeba* C. & B., 1895, is analogous to the hypothetical Genus *A* Linnaeus, 1758, in Opinion 6, in that when proposed it contained two species, which we now know as *coli* and *blattae*. Casagrandi and Barbagallo did not indicate which was the type. Opinion 6 declares that when an author has removed one of the two species to another monotypic genus, leaving only one species in the first genus, he is to be construed as having fixed the type of the first genus. Jordan's report in Opinion 99 follows the parallel exactly, crediting Chatton with having removed *coli* from *Entamoeba* C. & B. to the genus *Löschia*, thereby leaving *blattae* as the type of *Entamoeba*. If that is so, there is probably no doubt about the validity of the conclusion; but I think it is not true that Chatton really dealt with *Entamoeba* C. & B. in making the supposed division.

In every place in the three papers that Chatton wrote *Entamoeba* Leidy he was simply making a mistake for *Endamoeba* Leidy. Other authors before him who included *blattae*, with or without other amoebae, in *Entamoeba* C. & B. were also making a simple error; they should have used *Endamoeba* Leidy. The acts of Chatton and Lalung-Bonnaire were on *Endamoeba* Leidy, given by mistake as *Entamoeba* Leidy, but not corresponding to *Entamoeba* C. & B. Chatton (1910) grouped various amoebae in this "*Entamoeba* Leidy." Chatton and Lalung-Bonnaire (1912) did not agree with that grouping, and removed *coli* and other species from it, leaving only *blattae*. That made no change in the situation, except to restore it as it was originally. The revision was of the group concept authors had held of "*Entamoeba* Leidy" = *Endamoeba* Leidy, not of that genus itself, which was already attached to its type species.

The synonym argument in Opinion 99 depends upon crediting Chatton and Lalung-Bonnaire or Chatton with having comprehended *Entamoeba* C. & B. in what they did with "*Entamoeba* Leidy" = *Endamoeba* Leidy. Stiles' paragraph "d" in the argument, puts it: "Chatton's paper (1912, Bull. Soc. zool. France, p. 113) is to be interpreted as designating *blattae* as type of "*Entamoeba*" 1897 (= 1895) [emendation of *Endamoeba*, but obviously construed as identical with *Entamoeba*]." (Chatton and Lalung-Bonnaire had priority in this matter, and the page reference is wrong.) But Chatton's "emendation of" (rather, error for) *Endamoeba* was "*Entamoeba* Leidy," not "*Entamoeba* 1897 (= 1895)"; *Entamoeba* C. & B., 1895 was not an emendation, but a separately proposed word. Stiles' word "obviously" could have reference only to Chatton's opinion (1912) that the two words are orthographic variants, and therefore identical. Thus we return to the question of whether or not it is to be admitted that *Entamoeba* is a homonym of *Endamoeba*; and in consequence it appears to me that the whole argument of Opinion 99 stands or falls with the decision about the homonym question, in spite of the fact that the summary neglects that decision.

The summary of Opinion 99 presents the nomenclatorial treatment accorded *Entamoeba* C. & B. by Brumpt in 1913 as opposed to and invalidated by the prior treatment of that genus in the 1912 papers. On the contrary, however, it seems that Chatton and Brumpt had then exactly the same understanding of Casagrandi and Barbagallo's genus. In the historical account given above I quoted the statement by Chatton and Lalung-Bonnaire (1912, p. 142) that in 1897 Casagrandi and Barbagallo applied the name *Entamoeba* to intestinal amoebae of vertebrates, and the statement by Chatton (1912, p. 111) that those authors applied the name to a

human amoeba. Those are the only references in the 1912 papers to the correct and original use of *Entamoeba*. Brumpt, who in 1913 wrote "*Entamoeba* Leidy," had adopted the nomenclature of amoebae used by Chatton in 1910. In the footnote that was accepted by Stiles as constituting the type designation he simply gave a different wording of what the 1912 authors had pointed out regarding the amoeba for which the genus *Entamoeba* C. & B. had been proposed; but in that wording, and in printing the name "*E. hominis* synonyme de *E. coli*" he was more specific. Brumpt has more recently used both *Endamoeba* and *Entamoeba*; and it is likely that the 1912 authors would have used Casagrandi and Barbagallo's name for the species *coli* and other amoebae of vertebrates instead of *Löschia* except for the fact that they considered *Endamoeba* and *Entamoeba* to be orthographic variants. Despite the fact that Chatton and Brumpt evidently had the same understanding of *Entamoeba* C. & B., Stiles found it possible to give the interpretation that Chatton had designated *blattae* as its type before Brumpt designated *coli* as its type. Yet the only difference in the treatment the two authors gave the genus is that the former did not print the species name, whereas the latter did so. Brumpt, therefore, was not considered to have comprehended *Entamoeba* C. & B. in "*Entamoeba* Leidy," as regards type designation, whereas Chatton was considered to have done so. The interpretation given in this part of the argument in Opinion 99 is obviously greatly strained.

## CONCLUSION

Opinion 99 of the International Commission on Zoological Nomenclature does not constitute proof that *Entamoeba* Casagrandi & Barbagallo, 1895 cannot be used as a generic name. Its argument rests on two points: that *Entamoeba* is a homonym of *Endamoeba*; and that *blattae* is the type species of both, so that *Entamoeba* falls also as a synonym of *Endamoeba* Leidy, 1879. The latter point, which is the only one brought out in the summary of Opinion 99, is not acceptable: it rests on the interpretation that *Entamoeba* is a homonym of the earlier name. The Opinion asserts, but does not demonstrate, that it is a homonym; and there is nothing elsewhere in the Rules or Opinions that warrants the assertion. It is appropriate to place the species *coli* and *blattae* in separate genera; and it is considered that *Entamoeba* Casagrandi and Barbagallo, 1895, is available as a generic name for *coli* and congeneric species at the same time that *Endamoeba* Leidy, 1879, is used for *blattae* and congeneric species.

## REFERENCES

- ALEXEIEFF, A. 1912 Sur les caractères cytologiques et la systématique des Amibes du group *limax* (*Naegleria* nov. gen. et *Hartmannia* nov. gen.) et des Amibes parasites des vertébrés (*Proctamoeba* nov. gen.). Bull. Soc. Zool. France 37: 55-74.
- BRUMPT, E. 1913 Précis de parasitologie, 2. éd. Paris, Masson et Cie.
- 1922 Idem, 3. éd.
- 1927 Idem, 4. éd.
- 1936 Idem, 5. éd.
- CASAGRANDE, O. AND BARBAGALLO, P. 1895 Ricerche biologiche e cliniche sull' *Amoeba coli* (Lösch). Seconda ed ultima nota preliminare. Bol. Accad. Gioenia Sc. Nat. Catania, n.s., fasc. 41: 7-19.
- AND ——— 1897 *Entamoeba hominis* s. *Amoeba coli* (Lösch). Studio biologico e clinico. Annali d'Igiene sperimentale 7: 103-166.
- CHATTON, E. 1910 Essai sur la structure du noyau et la mitose chez les Amœbiens. Faits et théories. Arch. Zool. Expér. et Gén. (5) 5: 267-337.
- 1912 Sur quelques genres d'Amibes libres et parasites. Synonymies, homonymie, impropriété. Bull. Soc. Zool. France 37: 109-115.



- AND LALUNG-BONNAIRE 1912 Amibe limax (Vahlkampfa n. gen.) dans l'intestin humain. Son importance pour l'interprétation des amibes de culture. Bull. Soc. Path. Exot. 5: 135-143.
- CLEVELAND, L. R. AND SANDERS, E. P. 1930 Encystation, multiple fission without encystment, excystation, metacystic development, and variation in a pure line and nine strains of *Entamoeba histolytica*. Arch. Protistenk. 70: 223-266.
- CRAIG, C. F. 1911 The parasitic amoebae of man. Philadelphia, Lippincott.
- 1926 A manual of the parasitic protozoa of man. Philadelphia, Lippincott.
- 1944 Review of Kudo: Manual of human protozoa. Am. J. Trop. Med. 24: 330.
- DOBELL, C. 1919 The amoebae living in man. A zoological monograph. New York, Wm. Wood & Co.
- 1938 Researches on the intestinal protozoa of monkeys and man. IX. The life-history of *Entamoeba coli*, with special reference to metacystic development. Parasitology 30: 195-238.
- International Commission on Zoological Nomenclature. 1910 Opinion 6. In case of a genus *A* Linnaeus, 1758, with two species, *Ab* and *Ac*. Smithsonian Publication 1938: 7-9. Reprinted with editorial notes 1943 Opinions and Declarations rendered by the Inter. Com. Zool. Nomen. 1: 127-138.
- International Commission on Zoological Nomenclature. 1928 Opinion 99. *Endamoeba* Leidy, 1879, vs. *Entamoeba* Casagrandi and Barbagallo, 1895. Smithsonian Misc. Collect. 73(5): 4-8.
- International Commission on Zoological Nomenclature. 1943 Opinion 147. On the principles to be observed in interpreting Article 34 of the International Code in relation to the rejection, as homonyms, of generic and subgeneric names of the same origin and meaning as names previously published. Opinions and Declarations rendered by the Inter. Com. Zool. Nomen. 2: 123-132.
- KUDO, R. R. 1939 Protozoology. Springfield, Thomas.
- 1944 Manual of human protozoa. Springfield, Thomas.
- LEIDY, J. 1879a Fresh-water rhizopods of North America. Rep. U. S. Geol. Surv. of Territories 12: i-xi, 1-324.
- 1879b On *Amoeba blattae*. Proc. Acad. Nat. Sc. Philadelphia 31: 204-205.
- MEGLITSCH, P. A. 1940 Cytological observations on *Endamoeba blattae*. Illinois Biol. Monogr. 17 (4): 1-146.
- MORRIS, S. 1936 Studies of *Endamoeba blattae* (Bütschli). J. Morphol. 59: 225-263.
- PEARSE, A. S. 1942 Introduction to parasitology. Springfield, Thomas.
- REICHENOW, E. 1928 Doflein's Lehrbuch der Protozoenkunde, ed. 5, II. Teil. Jena, Fischer.
- SCHAUDINN, F. 1903 Untersuchungen über die Fortpflanzung einiger Rhizopoden. Arb. K. Gsndhsamte. 19: 547-576.
- STILES, C. W. AND BOECK, W. C. 1923 The nomenclatorial status of certain protozoa parasitic in man. Bull. Hyg. Lab. U. S. Pub. Health Serv. 133: 92-183.
- AND HASSALL, A. 1925 Key-catalogue of the protozoa reported for man. Bull. Hyg. Lab. U. S. Pub. Health Serv. 140: i-iv, 1-63.
- WENRICH, D. H. 1940 Nuclear structure and nuclear division in the trophic stages of *Entamoeba muris* (Protozoa, Sarcodina). J. Morphol. 66: 215-239.
- 1944 Studies on *Dientamoeba fragilis* (Protozoa). IV. Further observations, with an outline of present-day knowledge of this species. J. Parasitol. 30: 322-338.
- WENYON, C. M. 1926 Protozoology. New York, Wm. Wood & Co.



# THE STORAGE OF GLYCOGEN IN THE TEMNOCEPHALOIDEA

WILFRED FERNANDO

University of Ceylon, Colombo, Ceylon

## INTRODUCTION

The storage of glycogen in the body of the TEMNOCEPHALOIDEA has not so far been studied and the purpose of the present paper is to fill in this gap in our knowledge of carbohydrate metabolism in this group of PLATYHELMINTHES. The work was done entirely on adult animals, no attempt was made to study the distribution of glycogen during embryonic development.

I wish here to acknowledge my indebtedness to Prof. A. Kandiah and Dr. Eric Fonseka for the privilege of carrying out this work in the Department of Chemistry of the University of Ceylon and also to Prof. A. W. Mailvaganam for helpful suggestions. To Dr. Bruce Baptist of the Department of Agriculture, Peradeniya, I am indebted for the material which made possible the completion of the work. I must thank Mr. P. A. S. Perera of the Department of Physics for technical assistance.

## MATERIAL AND METHODS

The work was done on two representatives of the TEMNOCEPHALOIDEA; *Caridinicola indica*, which measures when preserved, about 0.6 mm. and *Monodiscus parvus* which measures about 0.3 mm. Specimens were obtained from fresh-water prawns at different seasons of the year from the following places in Ceylon—Anuradhapura, Kandy, Karawanella, Jaffna and Veyangoda.

As it was not considered safe to rely on any one method, several methods were employed to demonstrate the presence of glycogen in the body of these worms. In all more than one hundred specimens were sectioned and stained.

The following fixatives were used: Absolute alcohol-formol; Carnoy; Bouin-Allen (Pasteel's method); Chromo-osmic-acetic (Zieglwallner's method); and 10% formaldehyde saturated with dextrose (Neukirch's method).

The material was embedded by the paraffin method and by the celloidin-paraffin method. Sections were cut at  $5\mu$  to  $7\mu$ .

In staining, Best's carmine was found most satisfactory and results obtained from it were checked by the iodine method, Bauer's fuchsin—sulphurous acid method, and Fischer's tannin—gentian violet method. As controls, slides were digested with saliva for one hour at  $37^{\circ}$  C and stained.

## GLYCOGEN STORAGE IN *Caridinicola indica*

The glycogen in the outer epithelium of the body divides it into two layers—an outer or superficial layer which is free of glycogen and, next the basement membrane, an inner layer which has a great concentration of glycogen in the form of granules.

The basement membrane stains very deeply, indicating by its intensity a great concentration of the carbohydrate. It was not possible to identify in what form glycogen is stored here.

The connective tissues which form the greater part of the parenchyma contain glycogen in the form of fine granules and rodlets. A small quantity of glycogen is stored in the parenchymatous cells in the anterior and middle regions of the body. The intercellular spaces contain a glycogen-rich fluid, the granules being less prominent. It is possible that in these intercellular spaces the carbohydrate is in the process of transference to the different parts of the body.

The muscles are a very important place for the storage of glycogen. Those investing the reproductive organs contain large quantities and stain very intensely. Of the muscles of the body wall, the diagonal muscles in the ventral region of the body and the circular muscles at the anterior region have little glycogen, but elsewhere glycogen is present in the form of granules in the plasma of the muscle cells. The longitudinal muscles in every part of the body contain large quantities of glyco-

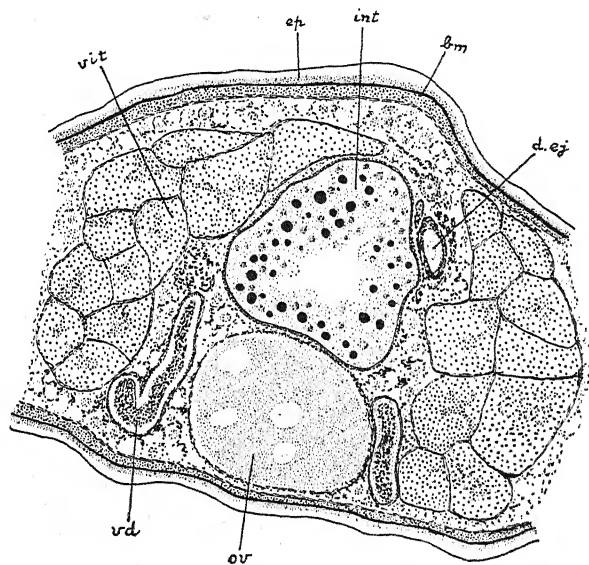


FIG. 1. Transverse section of *Caridinicola indica* passing through the region of the germarium. (Leitz ocular 16, obj. 6L) (Carnoy: Best's carmine). bm, basement membrane; d.ej, ductus ejaculatorius; ep, body epithelium; int, intestine; ov, germarium; vd, vas deferens; vit, vitelline gland.

gen, but the size of the muscle cells did not allow a very detailed study of the distribution.

The muscular posterior suckers have glycogen deposits, but the smaller, anterior suckers at the base of the tentacles are glycogen-free. On the other hand, the tentacles themselves contain glycogen granules. Numerous glands are present in the posterior suckers and in the tentacles and in these, glycogen is stored in the form of deeply staining granules or rodlets.

In the alimentary canal, the muscular pharynx (Fig. 3, ph) has glycogen deposited in the muscle fibres in the form of granules; at the posterior end of the pharynx these glycogen granules form a very characteristic, compact mass, contrasting that region sharply from the rest of the alimentary canal. The pharyngeal glands, which lie on the side of the pharynx, contain much glycogen. In the epithelial cells of the intestine, glycogen is present in the form of granules and also

as rounded masses inside vacuoles. The appearance of these glycogen masses in vacuoles in the intestinal epithelium is interesting (Fig. 1, int.). Some of them stain red, the others stain very lightly (pink), as compared with the glycogen in the muscles and it appears possible that with the absorption of food material, glycogen is synthesized in the epithelial cells and transported by way of the vacuoles to the outside and thence through the intercellular spaces of the parenchyma to the various regions of the body. The thin muscular coat investing the intestine, like other muscles in the body, has a deeply staining glycogen deposit.

Throughout the excretory system glycogen is present, distributed diffusely in the plasma of the excretory tubules. The staining is light in the tubules, but in the two excretory vesicles (Fig. 2) lying at the sides of the pharynx, the glycogen stains deeply and granular deposits can be seen. In these vesicles glycogen is deposited not only in the wall (as in the case of the excretory tubules) but also inside the lumen.

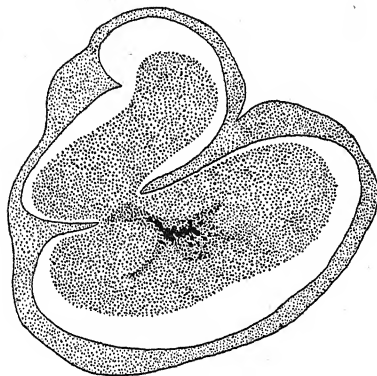


FIG. 2. Excretory vesicle of *Caridinicola indica* showing glycogen inside the vesicle and in the wall. (Leitz ocular 25, obj. 9A) (Carnoy: Best's carmine).

In the nervous system (Fig. 3) glycogen is found in the cerebral mass or brain in the form of fine, lightly staining granules and these granules can be traced to the nerves just as they issue out from the brain. More posteriorly the fine nerves are more or less obscured in the parenchymatous tissue so that it is not possible to state definitely whether glycogen is present throughout the nerves or not.

The muscular sheaths of the reproductive apparatus stain more intensely than do the muscles of the body wall, thereby indicating a more concentrated deposition of glycogen.

In the germarium (Fig. 1, ov), except for the very young oöcytes which are glycogen-free, the rest contain glycogen in the form of minute granules uniformly distributed in the cytoplasm. The epithelial investment of the germarium has no glycogen. The reservoir, into which the germarium opens, is a vesicle containing spermatozoa and yolk cells. Its epithelium has no glycogen, but the mass of spermatozoa found inside the lumen give a very dark glycogen stain. The function of the reservoir has been discussed in a previous paper (Fernando, 1934) and now it is apparent that the surplus spermatozoa are absorbed by the intestinal cells by way of the reservoir canal and the carbohydrate is passed into the body. The rest of the female apparatus consists of the oviduct, ootype and the atrium. In these, there is

general agreement in that the epithelium is always free of glycogen but the spermatozoa which travel along the female duct give the glycogen reaction. The unicellular glands which open into the oviduct throughout its entire length, contain glycogen granules which stain very deeply. Control slides, digested with saliva and stained by Best's carmine, do not take up the stain, so that it is evident that these glands contain glycogen.

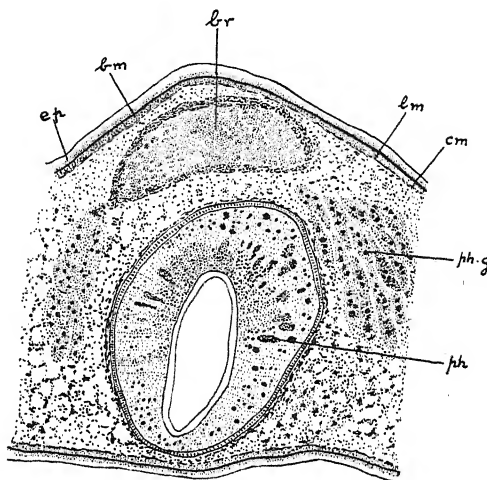


FIG. 3. Transverse section of *Caridinicola indica* passing through the brain and the pharynx. (Leitz ocular 16, obj. 6L) (Carnoy: Best's carmine). br, brain; cm, circular muscles; ep, body epithelium; lm, longitudinal muscles; ph, pharynx; ph.g, pharyngeal gland.

The vitelline glands contain glycogen (Fig. 1, vit). The membrane investing the follicles has rich deposits of glycogen granules; in the vitelline cells it is deposited as very small, uniformly distributed granules, which in some places become more concentrated and appear dark.

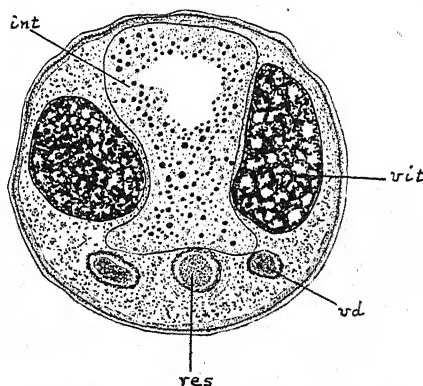


FIG. 4. Transverse section of *Monodiscus parvus* passing through the reservoir. (Leitz ocular 16, obj. 6L) (Carnoy: Best's carmine). int, intestine; res, reservoir; vd, vas deferens; vit, vitelline gland.

The envelope of the testes contains glycogen, but the developing sperm cells are glycogen-free. The mature spermatozoa, however, have a rich deposit of glycogen. The bundles of spermatozoa inside the testes give a very sharp contrast to the re-



maintaining unstained portions. The vasa deferentia and vesicula seminalis contain glycogen-staining spermatozoa. The epithelial parts of the vasa deferentia and the chitinous portion of the ductus ejaculatorius do not contain glycogen. The muscular sheaths throughout the male reproductive apparatus contain glycogen.

#### GLYCOGEN STORAGE IN *Monodiscus parvus*

The distribution of glycogen in *Monodiscus parvus* resembles closely that of *Caridinicola*, but with few differences. The glands of the posterior sucker and of the tentacles give a more intense stain than in *Caridinicola*. There is more glycogen in the alimentary canal of *Monodiscus*; this glycogen is present as granules and in vacuoles uniformly distributed in the intestinal epithelium. The glycogen vacuoles here are comparatively larger and more numerous. The brain contains glycogen but the excretory system of *Monodiscus* seems to be glycogen-free. In the reproductive system, *Monodiscus* resembles very closely *Caridinicola* in regard to the distribution of glycogen, but the vitelline glands of the former has a richer deposit which stains very intensely (Fig. 4). In brief, although *Monodiscus parvus* is a smaller worm than *Caridinicola indica*, glycogen is stored in comparatively greater quantity than in the latter (judging by the intensity of the stain).

#### DISCUSSION

The closest allies of the TEMNOCEPHALOIDEA are the TREMATODA and there is only one paper dealing with the distribution of glycogen in that group (Ortner-Schoenbach, 1913). The differences in regard to glycogen storage in the two groups may be mentioned. While the intestinal epithelium of trematodes is glycogen-free, that of temnocephalids contains glycogen, and in this the latter resemble the nematode *Ascaris* (Kemnitz, 1912). The trematodes are reported to lack glycogen in the nervous and excretory systems, but in the presence of glycogen in these two systems, the TEMNOCEPHALOIDEA resemble the ACANTHOCEPHALA as reported by von Brand (1939). The absence of glycogen in the excretory system of *Monodiscus* is noteworthy, but at present no explanation can be offered, nor can we explain why *Monodiscus* stores a relatively larger quantity of glycogen than does *Caridinicola*, which is a larger worm living together with the former on fresh-water prawns.

#### SUMMARY

1. Glycogen is stored in almost all the organs of the TEMNOCEPHALOIDEA.
2. It is absent or found in negligible quantities in the outer layer of the body epithelium, in the diagonal muscles and in the epithelial lining of the reproductive ducts.
3. The nervous system stores glycogen.
4. Glycogen is found in the excretory system of *Caridinicola* but not in *Monodiscus*.

#### REFERENCES

- BRAND, TH. VON. 1939 Chemical and morphological observations upon the composition of *Macracanthorhynchus hirudinaceus* (Acanthocephala). J. Parasitol. 25: 329-342.
- BRESSLAU, E. AND REISINGER, E. 1933 Temnocephalida: in Kükenthal's Handbuch der Zoologie.



- ERHARD, H. 1911 Glykogen in Nervenzellen. Biol. Zentrbl. 31: 472-475.
- FERNANDO, W. 1934 Studies on the Temnocephaloidea 1 & 2. Proc. Zool. Soc. Lond. 1934: 251-258, 827-850.
- KEMNITZ, G. v. 1912 Die Morphologie des Stoffwechsels bei *Ascaris lumbricoides*. Arch. f. Zellforsch. 7: 463-603.
- ORTNER-SCHOENBACH, P. 1913 Zur Morphologie des Glykogens bei Trematoden und Cestoden. Archiv. Zellforsch. 11: 413-449.
- VIALLI, M. 1927 La morfologia e la funzione del glicogeno in alcuni Vermi. Atti Soc. Ital. Sc. Natural. 66: 61-74.

DESCRIPTIONS OF TWO NEW SPECIES, *PARATRICHOBIOUS*  
*ANDUZEI* AND *NYCTERIBOSCA* *FRANCLEMONTI*  
(STREBLIDAE, DIPTERA, PUPIPARA)

ROBERT MATHESON

Jobling (1936) divided the family STREBLIDAE into four sub-families,—the NYCTERIBOSCINAE, the ASCODIPTERINAE, the TRICHOBIINAE, and the STREBLINAE. The genus *Paratrichobius* is placed in the TRICHOBIINAE. In 1939b Jobling described the known species of *Paratrichobius*—the type species *P. longicrus* (Ribeiro, 1907) and redescribed *Speiseria dunni* Curran as *P. dunni* (Curran). In the same publication he places the genus *Speiseria* Kessel (1925) as a synonym of *Synthesiostrebla* Townsend (1913). At the present time the genus *Paratrichobius* contains only the two above mentioned species. *P. longicrus* is reported from bats in Brazil, Paraguay and Mexico; *P. dunni* from bats in Panama. Some time ago I received three Streblids collected from bats (species not recorded) at San Estaban, Venezuela, by P. Anduze. These prove to be the third species for this genus.

*Paratrichobius anduzei*, new species

*Female*: Length 3.36 mm from tip of palps to tip of wings. Head strongly sclerotized with numerous stout, curving spines on the dorsal surface; eyes present, each consisting of eight facets; palps (Fig. 1, d) broadly rounded with one very long spine at outer apical point, several shorter ones and numerous short, setae-like spines; antennae present. Ventral surface of the head with a row of stout spines on each side of the rostrum membrane. Theca of labium cordiform, with four strong setae in front and a few scattered smaller ones (Fig. 1, e).

Prescutum (Fig. 1, a) somewhat pointed in front with a shallow indentation at the point of origin of the median suture; near the anterior margin is a row of short, stout spines; each antero-lateral corner with four or five stout, short spines; each lateral margin with a row of short, stout spines; area in front of transverse suture with small, scattered setae and a row of stouter setae in front of transverse suture. The transverse suture appears incomplete near the center and is not joined by the median suture. Scutum with numerous scattered small setae and a row of stout, spine-like setae just in front of the scutellum. Scutellum with sinuous posterior border and with four spines, the two posterior stout and very long; the anterior lateral in position and comparatively small. Legs stout; hind legs nearly three times as long as forelegs. Femora of prolegs each with a diagonal row of stout, spine-like setae and several smaller ones near them (Fig. 1, c); numerous short setae scattered over the femora; front tibiae with short setae and four long spines on outer margin.

Wing of female 2.6 mm in length; width at anterior cross-vein 0.8 mm.

Sternum narrowed in front and projects between and below the front coxae (Fig. 1, b) and is clothed with numerous, short, stout setae except the posterior margin which is provided with a row of stout spines. Ventral and lateral membranous parts of the abdomen with numerous small setae. The posterior border of tergite 1+2 interrupted in the middle and with a few fine setae; lateral borders enlarged and bear numerous, recurved, stout spines. Apical cone of abdomen with numerous long setae.

*Male*: The male closely resembles the female. The abdomen is more cylindrical and terminates in a long cone within which lie the genitalia.

*Holotype*: Female; paratypes, two males. Host, bats (species not recorded); collected at San Estaban, Venezuela, by P. Anduze on December 19, 1939. Types and paratypes in the collection of the Department of Entomology, Cornell University. I take pleasure in dedicating this species to the collector.

This species is easily distinguished from the two previously described species by the arrangement of the spines on the femora of the forelegs, by the shape of the mesonotum and the arrangement of its clothing setae, by the arrangement of the

spines on the scutellum, by the shape of the sternum and the abdomen with its much larger and stouter spines on tergite 1 + 2.

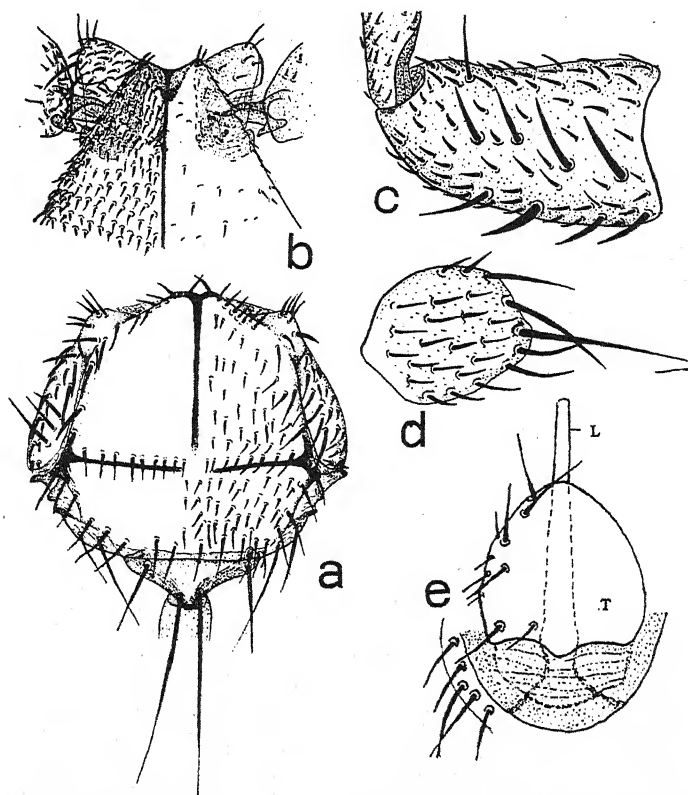


FIG. 1. *Paratrichobius anduzei*. a. Dorsal view of the thorax; the clothing setae are shown on only one side. b. Anterior part of the ventral surface of the thorax (Sternopleura of Jobling) projecting between and over the coxae. c. Femur of the foreleg showing the arrangement of the stout spines. d. Ventral view of the palpus. e. Ventral view of portion of head to show the theca and labella. L, labella; T, theca.

#### THE GENUS *Nycteribosca* SPEISER

Jobling (1934) monographed this genus, describing seven new species and re-describing the other eight known species; he was unable to place *N. diversa* Fraenkel. Since then the same author (1934, 1936a, 1936b) has described three new species—*N. minuta* from the Russell Islands, Solomon Islands; *N. bequaerti* from Tanganyika, Africa; and *N. scutellaris* from the Fiji Islands. Recently I received five specimens taken, presumably, from bats on Guadalcanal Island or the immediate vicinity by John Franclemont. This proves to be a very distinct species and is here described.

#### *Nycteribosca franclemonti*, new species

*Female*: Length 3.17 mm (from tip of palps to tip of wing). Head (Fig. 2, d) not darker than the body; eye distinct, large but not projecting laterally; each laterovertex with spines, nearly all of the same size and curving; a group near apex stouter and longer; mediovertex broad; postvertex a perfect circle enclosing two to four spines (three in the type female; four in the male); occiput with small setae behind the postvertex; sides of the head with strong but shorter spines; ventral surface of the head with stout spines. Palps prominent, longer than broad,

each with three long spines and many shorter ones. Antenna prominent, the arista stout and bears four branches. Theca of the labium nearly as broad as long, with many small setae.

Thorax (Fig. 2, a) circular as viewed from above, as broad as long. The mesonotal suture fades out near the center. The mesonotum is densely clothed with long, spiny setae; those on the anterior half are about one-third to one-half as long as those on the posterior half. The scutellum is evenly rounded behind and bears numerous long setae; a row of much shorter setae along the posterior border. Ventral surface of thorax (Fig. 2, b) clothed with short setae. Legs are all strong and armed with stout setae on the dorsal surface; ventral surface with short, hair-like setae. Wing measures 2.24 mm in length; width at the anterior cross-vein 0.93 mm. Venation as shown in Fig. 2, c. Veins 3 and 4 diverge towards the apex of the wing.

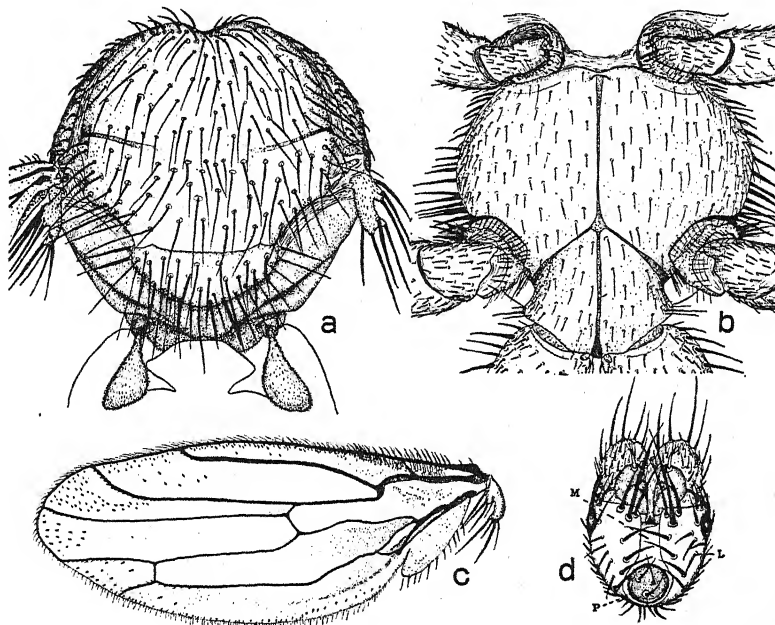


FIG. 2. *Nycteribosca franclemonti*. a. Dorsal view of the thorax. b. Ventral view of the thorax. c. The wing. d. Dorsal view of the head. L, laterovertex; M, mediovertex; P, postvertex.

Abdomen stout with dense masses of long hairs on each side of the bare part; each hair appears as if a series of fine parallel wires were twisted to a fine point. Tip of abdomen broadly conical; near the middle of cone on dorsal surface are four long setae; ventral surface with four long setae near middle and two before apex. Ventral surface of abdomen densely clothed with short stout setae.

The male is as large as the female and the only recognizable difference is the structure of the tip of the abdomen which bears the genitalia.

*Holotype*: Female; paratypes, 1 male and 3 females. Host a large bat. From Guadalcanal Island. J. Franclemont, collector. All specimens in the collection of the Department of Entomology, Cornell University.

This species runs to the group *pretiosa*, *amboinensis* and *surcoufi* in the key by Jobling (1934). It is readily separated from them by the circular postvertex, the characteristic setae of the scutum and scutellum and the density and length of the hairs of the abdomen. In the female the apical cone of the abdomen is different.

## REFERENCES

- CURRAN, C. H. 1935 New species of Nycteribiidae and Streblidae (Diptera). Amer. Mus. Novit. No. 765.
- JOBLING, B. 1934 Revision of the genus *Nycteribosca* Speiser (Diptera, Pupipara, Streblidae). Parasitology 26: 64-97.
- 1936a A new species of the genus *Nycteribosca*, with notes on *Nycteribosca minuta* Jobling. Proc. Roy. Entom. Soc. London (B) 5: 177-178.
- 1936b A revision of the subfamilies of the Streblidae and the genera of the subfamily Streblinae including a redescription of *Metelasmus pseudopterus* Coquillett and a description of two new species from Africa. Parasitology 28: 355-380.
- 1939a On the African Streblidae including the morphology of the genus *Ascodipteron* Adens. and a description of a new species. Parasitology 31: 147-164.
- 1939b On some American genera of the Streblidae and their species, with the description of a new species of *Trichobius*. Parasitology 31: 486-497.
- KESSEL, QUINTA C. 1925 A synopsis of the Streblidae of the world. J. New York Entom. Soc. 33: 11-33.



# EXPERIMENTAL INFECTION OF SOUTHERN CALIFORNIA MOSQUITOES WITH *WUCHERERIA BANCROFTI*

OLIVER K. SCOTT, Lt., (MC), USNR, CHARLES S. RICHARDS,  
MA PhM1/c, AND ELWOOD A. SEAMAN, PhM2/c

Numbers of military personnel have returned from the South Pacific Island area showing early stages of infection with *Wuchereria bancrofti*. Practically none of these men has ever shown microfilariae in the blood stream. Since their exposure was short, it is very doubtful that any of them will ever have demonstrable microfilariae in their peripheral blood stream. However, it is of some interest to determine which, if any, of the mosquitoes of hitherto uninfected parts of the country could become carriers of this disease. In this investigation, all the common mosquitoes of the San Diego, California, area were tested for transmission. This was made possible by obtaining an 18-year-old Negro Navy mess attendant, born in Frederickstad, St. Croix, Virgin Islands. He had spent all his life in Frederickstad except for parts of 6 years which were spent in San Juan, Puerto Rico, until he entered the Navy in November, 1943. At that time it was noted there were microfilariae in a night blood smear. At the time of these examinations there were up to 20 microfilariae per night thick smear. He denied ever having had any symptoms referable to filariasis.

Adult mosquitoes of seven different species were caught in the neighborhood of San Diego, and two species, *Aedes taeniorhynchus* and *Culex stigmatosoma*, were hatched from pupae. All were exposed to the infected subject at approximately 2200 (i.e., 10 P.M.), but three species would not feed (see Table I).

TABLE I. Results of dissections of mosquitoes fed on blood containing microfilaria

Mosquito	Dissected on death of mosquito before 17th day after feeding		Dissected alive on 17th day or longer	
	Infected	Not infected	Infected	Not infected
<i>Culex erythrothorax</i> .....	14	11	18*	12
<i>Culex quinquefasciatus</i> .....	4	2	17†	3
<i>Culex tarsalis</i> .....	0	0	1‡	2
<i>Aedes taeniorhynchus</i> .....	10	28	0	4
<i>Anopheles maculipennis</i> .....	0	1	0	6
<i>Anopheles pseudopunctipennis</i> .....	0	1	0	0
<i>Culiseta incidens</i> .....		Would not feed on subject		
<i>Culiseta inornata</i> .....		Would not feed on subject		
<i>Culex stigmatosoma</i> .....		Would not feed on subject		

Total dissections—134.

\* Eight of these had larvae in the proboscis.

† Thirteen of these had larvae in the proboscis.

‡ This one had larvae in the proboscis.

It was found in those mosquitoes which became infected that the infective stage of the larva did not appear until the 15th to 16th day after the blood meal, thereafter mosquitoes were not sacrificed until the 17th day.

Received for publication, March 16, 1945.

(This work was done under the auspices of U. S. Navy Epidemiology Unit 80, Lt. Comdr. G. R. Underwood, Commanding.)

A list of species and number of mosquitoes and results after feeding are listed below. Summarized results are also shown in Table I; Figs. 1 and 2 show developmental stages of the larvae in these mosquitoes.

*Culex quinquefasciatus* Say (= *C. fatigans*):<sup>1</sup> 26 specimens fed on the subject were dissected. Six died before 17 days and of these 6, 4 had developmental stages of the larvae in the thorax. Of the 20 specimens that went through 17 days, 17 had the infective stage in the head or thorax and in 13 cases the mature larvae were seen in the proboscis itself. Three specimens were negative.

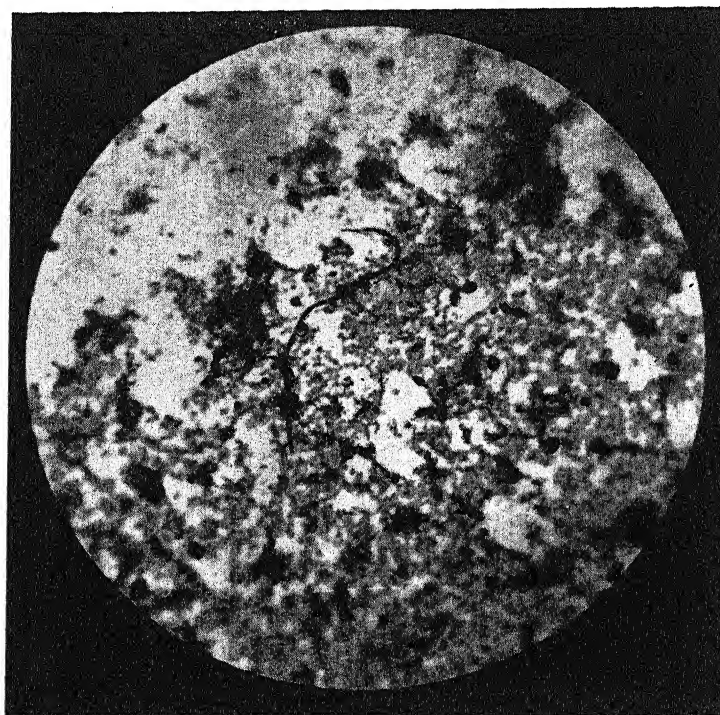


FIG. 1. Sheathless larvae in abdomen (outside stomach) of *Aedes taeniorhynchus*, 17 hours after feeding.

*Culex erythrothorax* Dyar: 56 specimens fed on the subject were dissected. Twenty-six died before 17 days. Of these 26, 14 had developmental stages of the larvae in the thorax. Of the 30 specimens that went through 17 days, 18 had the infective stage in the head or thorax and in 8 cases the mature larvae were seen in the proboscis itself. Twelve specimens were negative.

*Culex tarsalis* Coquillett: Due to the season of the year, late summer, only three specimens were obtained and fed. All of these survived 17 days and one had infective stages of the larvae in the thorax, head and proboscis. The other two specimens were negative.

*Aedes taeniorhynchus* (Wiedemann): 42 specimens fed on the subject were dissected. Thirty-eight died before 17 days and of these 38, only 10 had develop-

<sup>1</sup> Identification of this species made by the courtesy of Dr. Alan Stone of the U. S. National Museum, Washington, D. C., and Dr. W. C. Reeves, of the George Williams Hooper Foundation, San Francisco.

mental stages of the larvae in the thorax. The four that survived 17 days were all negative.

*Anopheles maculipennis freeborni* Aitken: Seven specimens fed were dissected. All specimens were uninfected and all but one survived for 17 days.

*Anopheles pseudopunctipennis* Theobald: This species would not feed except one specimen which did not become infected.

*Culex stigmatosoma* Dyar: Although very large numbers, several hundred, were exposed to the patient, none fed.

*Culiseta inornata* (Williston) and *Culiseta incidens* (Thomson), the other two common mosquitoes in this area, do not ordinarily feed on humans, and the specimens obtained did not feed on this individual.



FIG. 2. Larva in thorax of *C. quinquefasciatus*, 7 days after feeding.

#### CONCLUSIONS

If persons with circulating microfilariae were present in Southern California, the local mosquitoes *Culex quinquefasciatus*, *Culex erythrothorax*, and probably *Culex tarsalis* would be vectors of the Bancroftian filariasis. Because so many mosquitoes died under laboratory conditions the facts are inconclusive as to *Aedes taeniorhynchus*, but what evidence there is indicates that it would be a less efficient vector than the above-mentioned three species. *Anopheles maculipennis freeborni*, *Anopheles pseudopunctipennis* and *Culex stigmatosoma*, and the *Culisetas*, would be unlikely vectors.

*PAGURITHERIUM ALATUM* N. G., N. SP., AN ENTONISCIAN  
PARASITE OF *PAGURUS LONGICARPUS*

EDWARD G. REINHARD

Department of Biology, Catholic University of America

The ENTONISCIDAE constitute a very specialized family of isopod crustaceans (suborder EPICARIDEA), whose members are found only within the haemocoel of brachyuran and anomouran crabs, where they are invested by an integumental sheath that communicates with the exterior of the host. Until recently no representatives of this family had been reported from North America. In 1938 Pearse and Walker (1939) found two species in xanthid crabs from North Carolina and Prince Edward Island which proved to be new species of the genus *Cancrion*. In 1943 the writer discovered another entoniscid at Woods Hole, Mass. This parasite infects hermit crabs and represents a new species and new genus. Moreover, it is peculiar in communicating with the exterior not as others are reported to do, by way of the host's branchial chamber, but through an opening in the head or eyestalk of the crab.

The host of this entoniscid is *Pagurus longicarpus* Say, a littoral species extremely abundant in the Woods Hole region. All the crabs were collected from the beach fronting the Yacht Club in Great Harbor. Of some 4600 taken from this locality during August and September, 1943, 38 crabs were found to contain one or more adult female parasites, an infection rate of 0.8 per cent.

A hermit crab containing an adult entoniscid can be recognized with practice by the pale appearance of the abdomen and the scanty liver tissue. Often the eggs and embryos of the parasite are discernible through the semi-transparent integument of the host's abdomen. With the exception of a hundred or so apparently normal specimens the only crabs dissected were those showing external indications of the presence of parasites and therefore young stages were not encountered. A complete examination of all crabs to uncover those having the "asticot" stage would undoubtedly have raised the percentage of parasitism but the task would have been very arduous since the infection rate with all stages could not have exceeded 1.5 or 2 per cent.

The adult male and female parasites resemble most closely the males and females of the genus *Entoniscus*. Similarity to *Entoniscoides* is less marked. In important respects, however, this entoniscid differs from these and other known genera of the family and a new genus is required to contain it. This genus we propose to call *Paguritherium* (*Pagurus* + *therion*, a beast). The specific name *alatum* is in reference to the first pair of oostegites of the female which resemble great wings.

Cotypes of this new entoniscid have been deposited in the U. S. National Museum.

RELATION OF THE PARASITE TO THE HOST

The full-grown parasite is about two-thirds as long as the crab. The body proper with its enormously developed brood chamber occupies most of the available space in the abdomen of the host and its thin elongated pleon reaches forward



through the host's thorax into the most anterior portion of the head (Fig. 1.) Not only is its position reversed with respect to the host but also inverted since the parasite's dorsal surface is directed towards the ventral surface of the host.

In traversing the thorax the pleon passes beneath the heart, stomach and brain but since it is merely a slender tube the organs of the crab in this part of its body are not displaced or harmed. In the abdomen, however, where the bulk of the parasite is located, the hepatopancreas of the crab and its reproductive organs are greatly reduced. In some cases only by means of serial sections can any trace of the gonads be found.

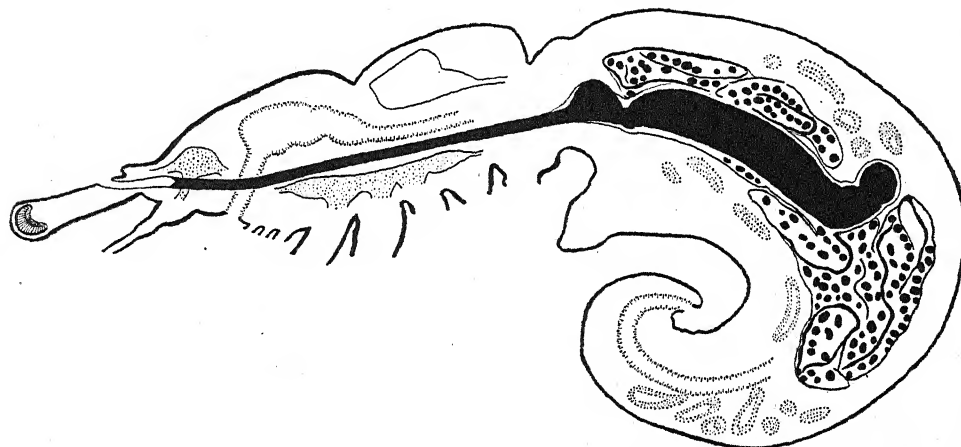


FIG. 1. Diagram of a hermit crab showing the parasite, *Paguritherium alatum*, in situ. Note its communication with the exterior through the wall of the crab's eyestalk. The parasite proper is represented in solid black. Surrounding it is the enclosing sheath and, to the right, the brood pouch of the parasite filled with embryos.

Throughout its entire extent the entoniscid is invested in a thin sac closely molded to the contour of its body. This sheath is derived from the hypodermis of the host and is seen distinctly as a separate entity only near its point of origin at a perforation in the body wall of the crab. There it forms a short hollow stalk since the tip of the parasite's pleon does not quite extend all the way to the aperture. The integumental sheath of entoniscids arises, as has been shown by Giard and Bonnier (1887) and others, at the time when the larval entoniscid punctures the integument of the crab to gain entrance into the host's body and spreads inwards progressively as the parasite penetrates further and grows larger. The sac thus formed shuts off the animal from direct contact with the crab's viscera but at all times retains its communication with the exterior at the original point of invagination.

In the case of all previously studied entoniscids this aperture occurs in the inner wall of the branchial chamber of the host. In the present animal, however, it lies in the head or eyestalk of the crab. The precise location was determined in ten infected crabs by careful dissection or by means of serial sections and gave results as follows:

In the corneal surface of the eyestalk .....	2
In the distal half of the eyestalk .....	3
In the proximal half of the eyestalk .....	1
At the base of the eyestalk .....	2
At the base of the second antenna .....	2



The aperture in the case of the present species is a somewhat oval opening measuring only 0.15 mm in greater diameter and about 0.10 mm in lesser diameter. The inturning of the hypodermis at the margins of this opening is accompanied by an inturning of part of the chitin layer which thus forms a ring over the inner surface of the sheath for a slight distance back from the opening. To this thickened portion Giard and Bonnier gave the name "casque" or "calyce chitineux." In the present entoniscid it is not as well developed as in the genera described by them. Its extent is only about 0.2 mm and its thickness about 10  $\mu$ .

*Paguritherium* new genus

*Female*.—Body slender and lacking ovarian processes; brood chamber open; four pairs of well-developed oöstegites and a rudimentary fifth pair; margins of oöstegites not deeply notched; oöstegite I the largest, consisting of a large ascending lamella folded laterally into an inner and outer plate, with descending lamella very short. Pleon long, practically uniform in diameter throughout, with five pairs of saber-like appendages; telson invaginated.

*Male*.—Antennules tuberculiform; pereopods stump-like and spinose anteriorly; telson unforked, linguiform and spinose posteriorly.

*Genotype*.—The species described below.

*Paguritherium alatum* new species

The Female (Figs. 2-4)

The cephalogaster is a swollen bilobed structure as in other ENTONISCIDAE, with antennules and antennae in the form of thickened pads fused lip-like around the mouth. The only prominent mouthparts are a pair of fleshy maxillipeds. Maxillae are lacking and the mandibles are extremely minute. A possible peculiarity of the cephalogaster of this species is the comparative narrowness of the hemispheres.

The thorax or perion, which is almost entirely filled by a highly developed ovary and the hepatopancreas, is a smooth narrow cylinder without ovarian processes. It bears the oöstegites which form the framework for a brood chamber of the open type. Over this framework is stretched the integumental sheath so that a capacious pouch is formed to hold the developing eggs and embryos.

Only the first four pairs of oöstegites are concerned in brood pouch formation. The two lamellae of each of these pairs overlap in the mid-ventral line and the posterior margins of oöstegites II and III also overlap the anterior margins of oöstegites III and IV, respectively. The lamellae are leaf-like in appearance, exhibiting a venation which usually takes the form of three main veins with subdividing branches. At their free margins they are crispate and have a few shallow indentations.

Oöstegite I consists of an ascending lamella of considerable length and a very short descending lamella. The ascending branch rises over the cephalic region like a great wing and is folded laterally upon itself so that a shorter outer leaf and a very much longer inner leaf are differentiated. A descending branch and possibly a transverse branch are present but both are quite rudimentary. This oöstegite is inserted near the upper end of the thorax and between it and the insertion of the next oöstegite, there is a conspicuous gap.

Oöstegite II consists of a single ascending lamella which extends upwards from about the middle of the thorax without quite reaching the cephalogaster. It is about two-thirds as long as the ascending branch of oöstegite I and somewhat narrower.

The third oöstegite is about half as long as its predecessor and the fourth is still shorter, but both diminish only slightly in breadth. They project ventrally also and are so close together that the latter is practically a lobe of the former. Oöstegite V is a narrow inconspicuous plate crowded out of position and folded against the dorsal surface of the thorax in front of the heart, thereby forming a pocket for the storage of sperm.

The pleon is sharply set off from the thorax and consists of five pleomeres and a telson. In the living animal it can be extended to a length exceeding that of the thorax but in preserved specimens it is always found strongly contracted, particularly in the region of somites III to V. At its junction with the thorax it is greatly dilated by the presence of the heart. Elsewhere it is a rather slender stalk-like structure of almost uniform diameter throughout, tapering only slightly at the posterior end. Each pleomere is provided with a pair of appendages in the form of pointed saber-like processes. The pleural lamellae, conspicuous structures in most ENTONISCIDAE, are here represented merely by a narrow membranous frill on the two proximal

segments. These segments are considerably longer than the others. The truncate telson is excavated at its distal extremity and crowned by a fringe of short marginal hairs.

The general color of the female is pale yellow, but the presence of mature embryos may give it a deep yellow or rusty appearance. The hepatopancreas has a red coloration and the oöstegites are whitish.

One of the larger specimens measured with brood pouch filled and enclosing sheath present had a total length of 15 mm including the pleon which measured about 5 mm. The greatest width at any one point was about 4 mm.

The female of this genus differs from *Etoniscus* Müller chiefly in having the anterior oöstegites greatly enlarged, with the second to the fourth decreasing in size, and the fifth pair rudimentary, while in *Etoniscus porcellanae*, the type species of the genus, all oöstegites are well developed, with the posterior pairs larger than the anterior ones.

#### The Male (Figs. 5, 6)

The male is found inside the brood chamber of the female, and, as a rule, one male accompanies each female.

The male attains a length of about 2 mm. The specimen drawn measured 1.8 mm. The body color is pale with a few dark brown chromatophores. In general shape of the body and particularly in the stump-like appendages it resembles the male of *Etoniscus porcellanae*, a Brazilian species described by Müller (1862). It differs notably, however, in the shape, size and position of the antennules, which, in *E. porcellanae*, form prominent, antero-lateral, quadrangular expansions.

The head of the male is short and blunt and has antennules in the form of knob-like protuberances that project forward without reaching the frontal margin. Each is surmounted by about 10 short curved setae. The antennae are represented only by an inconspicuous tuft of bristles in front of the eyes. The mandibles, which are moderately narrow and notched at the tip, are enclosed in a suctorial cone as is usual in the ENTONISCIDAE. The opening of this cone is somewhat ellipsoidal with an acute mid-lateral expansion on each side. The surface of the lower lip is smooth, not covered with scale-like markings as occur for example in *Pinnotherion* or *Entionella*. Maxillulae could not be detected. The maxillae appear to be small wing-shaped lamellae meeting in the mid-line. These are concealed below broader leaf-like plates which represent the maxillipedes.

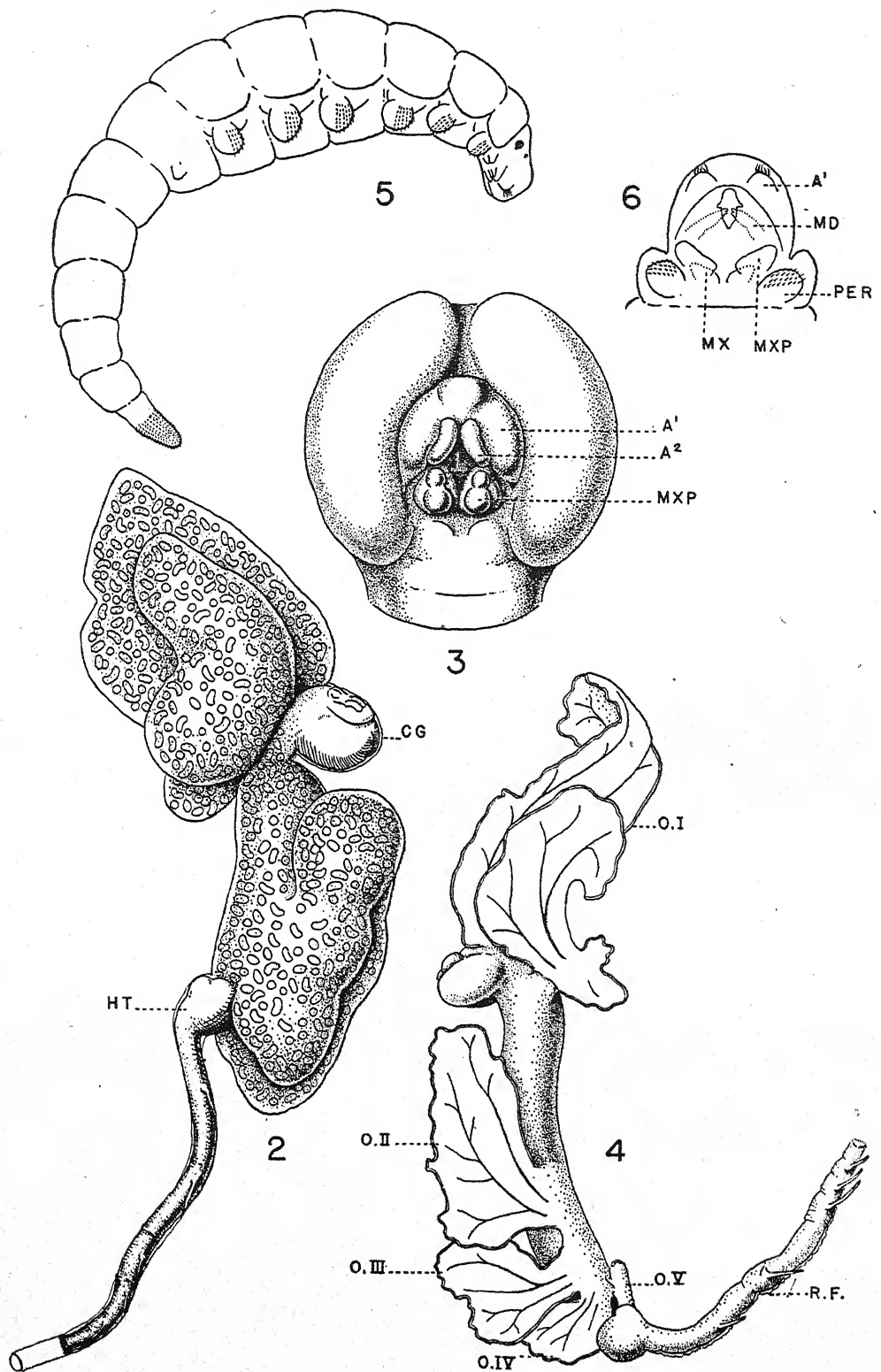
The rather plump thorax is composed of seven somites carrying six pairs of stump-like appendages on I to VI. These thoracopods (pereopods) are all alike except that they increase slightly in size to the fifth pair, the sixth and fourth being about equal. Each consists of a broad, fleshy basal segment and a rounded protopodite which is a single undivided knob. The protopodite is provided on its anterior half with rows of short spines. The seventh thoracic segment bears a pair of smooth lateral tubercles. Miyashita (1940) regards similar protuberances on the seventh somite of the male *Etoniscoides* as rudimentary pleopods but they lack the basal piece and in the present instance the spines of the other pereopods. It is evident that these are genital tubercles, homologous with the penis of *Priapion*.

The pleon consists of five segments and a telson, the latter tongue-shaped and undivided. The pleomeres are without appendages and diminish in size posteriorly. Spines are absent from the ventral side of the pleomeres but occur on the posterior half or posterior two-thirds of the telson.

#### DEVELOPMENT

In previously described ENTONISCIDAE, with the exception of *Etoniscus* and *Etoniscoides*, the embryos in the brood chamber are all of the same age. Embryos of diverse stages are present in one brood of *Etoniscus porcellanae* according to Müller (1862), and hatching occurs in the epicarid stage. A Japanese species of *Etoniscus* is reported by Miyashita (1940, p. 153 footnote) to have a brood consisting of three different sorts of embryos. In *Etoniscoides okadai* two kinds of embryos make up the content of the brood chamber and development is abbreviated so that the larva hatches in the cryptoniscan stage (Miyashita, 1940).

The present species is like *Etoniscus porcellanae* in that a mixture of eggs in early development and advanced larval forms are invariably found in the same



brood pouch. It appears that a second and often a third batch of eggs is released, after a considerable time interval, into the pouch which already contains the developing embryos of a previous laying, so that two or three widely different stages are encountered together. The free-swimming larval stage which escapes from the brood chamber is epicarid in form. It does not differ materially in structure from that of *Pinnotherion vermiforme* described and figured by Atkins (1933). When ready for emergence it makes its way into the space between the integumental sheath and the pleon of the parent and crawls out through the aperture in the body wall of the crab with which the sheath is in constant communication.

The round unsegmented ova vary in diameter from  $95\ \mu$  to  $115\ \mu$ . Segmentation results in an oval embryo about  $132\ \mu$  by  $95\ \mu$ . Young epicarid embryos measure from  $160\ \mu$  to  $180\ \mu$  in length and  $64\ \mu$  in dorso-ventral direction; older epicarids measure about  $227\ \mu$  in length and  $90\ \mu$  in breadth.

The last larval or cryptoniscan stage was not seen. All the cryptoniscan larvae found clinging to the surface of the host proved to be those of the bopyrid *Stegophryxus hyptius* with which *Pagurus longicarpus* is likewise parasitized at Woods Hole (Thompson, 1902; Reinhard, 1943).

#### SUMMARY

*Paguritherium alatum*, representing a new genus and species of parasitic isopods of the family ENTONISCIDAE, is described.

The genus is most closely related to *Entoniscus*, but differs from it, in the case of the female, by the greater enlargement of the anterior oostegites and the reduction of the posterior ones, while, in the male, the antennules do not form antero-lateral expansions.

Unlike other known entoniscids, this species communicates with the exterior through an opening in the head or eyestalk of the host.

The host is *Pagurus longicarpus* Say. Thirty-eight hermit crabs were found at Woods Hole, Mass., parasitized with adult stages, an infection rate of 0.8 per cent.

#### EXPLANATION OF FIGURES

##### Abbreviations

A <sup>1</sup> —antennules	O.I.—first oostegite
A <sup>2</sup> —antennae	O.II—second oostegite
CG—cephalogaster	O.III—third oostegite
HT—heart	O.IV—fourth oostegite
MD—mandibles	O.V—fifth oostegite
MX—maxillae	PER—pereopods
MXP—maxillipeds	R.F.—respiratory folds

FIG. 2. Adult female of *Paguritherium alatum* with embryos present in brood pouch and enclosing sheath intact.

FIG. 3. The cephalogaster of a female.

FIG. 4. Adult female stripped of enclosing sheath and contents of brood pouch. The oostegites are drawn only on the left side.

FIG. 5. Lateral view of a male of *Paguritherium alatum*.

FIG. 6. Ventral view of the head region of a male.

## REFERENCES

- ATKINS, D. 1933 *Pinnotherion vermiforme* Giard and Bonnier, an entoniscid infecting *Pinnotheres pisum*. Proc. Zool. Soc. London, 1933: 319-363.
- GIARD, A. AND BONNIER, J. 1887 Contributions à l'étude des Bopyriens. Trav. Sta. zool. Wimereux 5: 1-252.
- MIYASHITA, Y. 1940 On an entoniscid with abbreviated development, *Entoniscoides okadui*, n.g., n. sp. Annot. zool. Japon., 19: 149-157.
- MÜLLER, F. 1862 *Entoniscus porcellanæ*, eine neue Schmarotzerassel. Arch. f. Naturgesch. Jg. 28: 10-18.
- PEARSE, A. S. AND WALKER, H. A. 1939 Two new parasitic isopods from the eastern coast of North America. Proc. U. S. Nat. Mus. 87: 19-23.
- REINHARD, E. G. 1943 Epicaridean parasites of *Pagurus longicarpus* at Woods Hole. Anat. Rec. (Abst.) 87: 468.
- THOMPSON, M. T. 1902 A new isopod parasitic on the hermit crab. Bull. U. S. Fish Comm. 21: 53-56.



## METAMORPHOSIS OF THE FROG HOST AS A FACTOR IN CERCARIAL PENETRATION BY *GLYPHHELMINS QUIETA*\*

BY W. HENRY LEIGH<sup>1</sup> AND HARLEY J. VAN CLEAVE<sup>2</sup>

*Glyphelmims quieta* (Stafford, 1900) is a digenetic trematode of the family PLAGIORCHIDAE whose marita occurs in the intestine of frogs (Leigh, 1937, et al). Several species of snails of the genus *Physa* serve as the first intermediate hosts for the alternate generations of *Glyphelmims*. Cercariae liberated from the body of a snail become encysted as metacercariae in the skin of frogs (Leigh, 1937, and in press; Rankin, 1944). The worms gain access to the intestine of the definitive host only when the cast skin bearing the encysted metacercariae is devoured by the frog. Thus the frog serves both as second intermediate host and definitive host for *Glyphelmims quieta*. This unusual situation is made possible by the fact that a frog devours its own cast skin. Active cercariae ingested by tadpoles and frogs are apparently destroyed in the digestive tract for they have never been found encysted in the viscera.

Preliminary observations and experiments had demonstrated the fact that the cercariae of *G. quieta* refuse to penetrate and become encysted in the skin of tadpoles of the same species of frogs which serve as normal intermediate hosts following metamorphosis. Habits of the cercariae, which usually swim upward toward the surface-film, would tend to avoid natural contact with tadpoles but would facilitate contact with frogs swimming or resting at the surface.

In repeated attempts, tadpoles of *Rana pipiens* and *R. catesbeiana* were exposed to thousands of cercariae of *Glyphelmims* in glass dishes. The reaction was invariably different from that observed with frogs. Upon contacting the skin of a tadpole, the cercariae became attached and crawled about rapidly, never stopping to initiate the movements characteristically performed in attempting penetration. Their uninterrupted movements gave the impression that they were "seeking" suitable spots which they never found. During this period of rapid crawling the cercariae were attached by the suckers only and in numerous instances vigorous movements of the tadpole were observed to dislodge them.

The difference in response of the cercariae to skin of tadpole and metamorphosed frog suggested the problem of determining experimentally the conditions under which penetration became possible and an analysis of these conditions. A series of tadpoles of *Rana catesbeiana* was used in these experiments.

In two instances tadpoles which had begun metamorphosis and had attained the hind legs were exposed to large numbers of cercariae of *G. quieta*. Although the tail had begun to degenerate, it was still present. Cercariae became encysted in the skin of the legs and body but none penetrated the skin of the tail in either experiment. These experiments suggested that metamorphosis, as it affects cercarial penetration, does not affect the entire body simultaneously but is accompanied

Received for publication, May 11, 1945.

\* A contribution from the Zoological Laboratory, University of Illinois.

<sup>1</sup> Aviation Physiologist, United States Army Air Forces.

<sup>2</sup> University of Illinois, Urbana, Illinois.

## EXPLANATION OF PLATE

All figures were prepared as preliminary camera lucida sketches which were redrawn by Katharine Hill Paul, scientific artist in the Department of Zoology of the University of Illinois. Each line indicating magnification has the value of 0.05 mm.

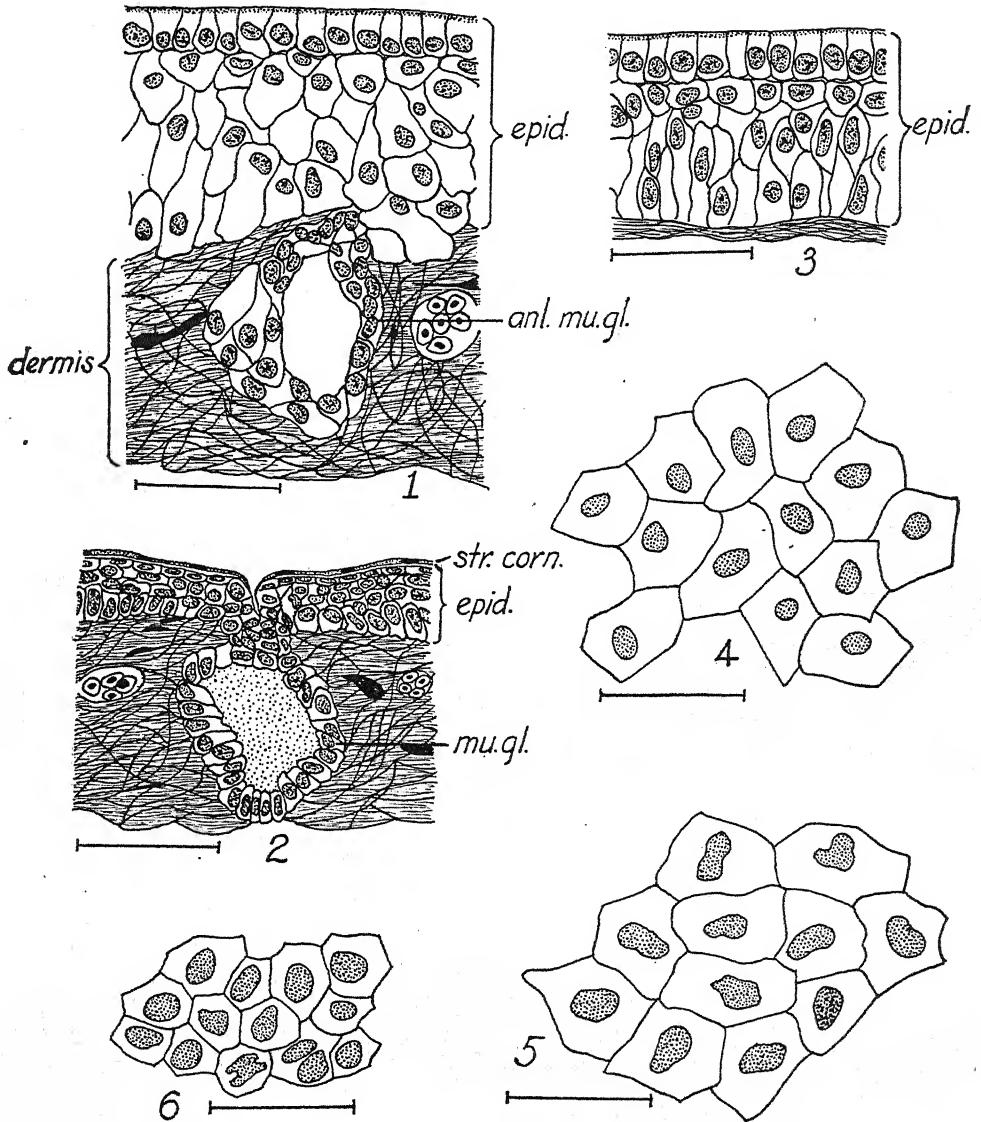
Integument of *Rana*

FIG. 1. Section through the body integument of a tadpole of *R. catesbeiana* before exposure to thyroid extract showing normal, unmetamorphosed larval skin. Note that the anlage of the mucus gland (anl. mu. gl.) has not yet acquired a duct. The external layer of the epidermis (epid.) has not yet become a stratum corneum.

FIG. 2. Section of the body integument of a tadpole of *R. catesbeiana* following exposure to thyroid extract. Note that the skin has undergone metamorphosis. The outer layer of cells (str. corn.) now forms a stratum corneum, the entire epidermis (epid.) has undergone transformation, and the mucus gland (mu. gl.) has attained connection with the surface.

FIG. 3. Section of tail of normal tadpole of *R. catesbeiana* showing unmetamorphosed epidermis (epid.).

by a series of progressive, coordinated morphological and physiological changes in the skin.

There is an extremely voluminous literature on normal and experimentally induced metamorphosis in AMPHIBIA, but most of this is concerned primarily with the conspicuous organic changes that mark the attainment of legs and loss of the tail. The histological changes accompanying metamorphosis of the tadpole are less obvious but are fully as significant. Microscopic examination of the skin reveals marked differences between that of the tadpole and the frog. The epidermis of the tadpole (Fig. 1) is much thicker than that of the frog (Fig. 2), chiefly because the component cells are larger. In the tadpole, cells of the outer layer are cuboidal to slightly columnar in form but upon metamorphosis the integument of the frog attains a covering layer of very flat, squamous epithelium designated as the stratum corneum. The large acinous glands, characteristic of the adult skin, are entirely lacking in the tadpole. In fact Wilder (1925) and Helff (1931) state that no anlagen of the acinous glands appear until just prior to the onset of metamorphosis. In the tadpole (Fig. 1) no ducts are formed to connect these anlagen with the external surface. One type of the acinous glands produces the mucus so distinctive of the skin of an adult frog. Although there are other minor differences between the skin of the tadpole and that of the frog the three here enumerated seem particularly significant: (1) change in the shape of the cells covering the body surface (Figs. 4, 5, 6), (2) attainment of gland openings, (3) the functioning of the mucus and other acinous glands. The differences here enumerated suggest that either structural or physiological changes in the skin might furnish the basis for explaining the difference in susceptibility to cercarial penetration. It seems probable that structural and physiological changes are both important.

That cercarial penetration of tadpole skin is not physically impossible is shown by the fact that in other species of XIPHIDIOCERCARIAE, fairly closely related to *Glypthelmins*, penetration of tadpole skin is accomplished regularly in carrying on the normal life cycle. Thus McCoy (1928), Talbot (1933), Byrd (1935) and McMullen (1935) have demonstrated life histories in which XIPHIDIOCERCARIAE pass through the skin of tadpoles and become encysted in deeper tissues. These observations would suggest that the stimulus or chain of stimuli leading to penetration may vary in closely related species of trematodes and that stimulation which will initiate penetration for one species may be ineffectual for another.

One set of observations seems to localize definitely the source of the stimulation to penetration within the skin of the metamorphosed frog. With only the cast skin from a metamorphosed frog in the container, cercariae have been observed to accomplish complete penetration from either side of the stratum corneum. Penetration of the cast-off skin was slower than normal penetration of the skin attached to the frog body. This might readily be explained by the fact that the detached skin allowed for lost motion more than the skin tightly drawn over the frog's body. The cercarial tail was dropped after penetration of the loose skin began and when the

FIG. 4. Surface view of a piece of cast skin from the body of an adult *R. pipiens*, showing relative size of cells and nuclei.

FIG. 5. Surface view of a piece of cast skin from the body of a tadpole of *R. catesbeiana* following exposure to thyroid extract.

FIG. 6. Surface view of a piece of cast skin from the body of a normally metamorphosed young individual of *R. catesbeiana*.

tailless cercaria fell through on the opposite side of the skin the stimulus for further attempt at penetration seemed lacking. There was no evidence of cyst formation following successful penetration of shed skin.

Since the functioning of the mucus glands is one of the most distinctive changes marking metamorphosis of the frog skin, a series of experiments was devised to see whether the mucus could be responsible for the stimulation of cercariae to penetration. There are no direct observations that would tend to indicate that penetration is by way of the openings of the acinous glands. In fact detailed observations by McMullen (1937: 236) upon the mechanics of penetration by another species of XIPHIDIOCERCARIAE stress the importance of the stylet as an excavating tool in piercing the body covering of a second intermediate host. Mucus scraped from the skin of a frog was spread on the body of a tadpole which was then subjected to cercariae of *Glythelmins*. The cercariae gave no evidence of attempt at penetration. It is probable that most of the mucus was washed from the skin of the tadpole when it was returned to the water.

If mucus is the agent responsible for initiating the penetration responses of *Glythelmins* cercariae there must be some specific substance other than the mucin present because the cercariae fail to penetrate the skin of salamanders, rich in mucus glands. Two series of experiments were carried on to test the potentiality of mucin as a stimulus to the cercariae. Mucin was extracted from the skin of *Rana pipiens* in 10% NaCl and was then precipitated in dilute acetic acid, washed and filtered. The dried extract was removed in thin sheets from the filter paper. These thin sheets, insoluble in water and about the thickness of the stratum corneum, were placed in water containing cercariae of *Glythelmins*. Many of the latter attached themselves temporarily but made none of the characteristic movements associated with the act of penetration. It is possible that some specific substance capable of influencing action of the cercariae was destroyed during the preparation of the mucin. Mucin from another source was likewise tested in a series of non-conclusive trials. NaOH was used to extract mucin from ground parotid glands of sheep. When this was precipitated by weak acetic acid and placed on the skin of tadpoles it produced no observable change in the indifferent response of the cercariae.

A tadpole that was undergoing normal metamorphosis in the laboratory was exposed to cercariae after both pairs of legs had appeared although much of the tail still remained. Cercariae penetrated the skin of the legs and body and became encysted although they did not attempt to enter the skin of the tail. The encysted metacercariae were evident in the body and leg skin that was shed by this individual, and on autopsy it was found that some cercariae had penetrated into the deeper layers of the skin and were not sloughed off with the molt. Microscopic examination of pieces of the shed skin showed the flattened external layer of cells and many openings of acinous glands distinctive of the skin of the adult frog.

In another instance of a normally metamorphosing tadpole the skin of the hind legs contained a number of encysted metacercariae although none could be found in the skin of either the body or the tail. These results suggest that the metamorphosis of the skin is progressive and that a new adult organ such as the leg has a skin characteristic of the adult before the skin of the larval body transforms.

In order to observe the effects of accelerated metamorphosis of tadpoles upon the reactions of cercariae, young tadpoles of *Rana catesbeiana* were treated with



thyroid extract. In from 7 to 10 days the treated tadpoles showed a marked change in skin texture (compare Figs. 1 and 2) and in susceptibility to cercarial penetration. The outer layer of columnar cells on the body proper was replaced by a squamous epithelial layer of cornified cells identical in appearance with the stratum corneum of the mature frog. Cells of the deeper layer of the skin became much reduced in size, and the nuclei occupied a larger volume of the cells. These histological changes took place in the thyroid-treated frogs before the front legs and other gross evidences of metamorphosis had made their appearance. The hind legs had just begun to develop and the intestine retained its much coiled condition when the treated tadpoles began to molt a layer of skin that had every appearance of the skin of an adult frog. Following this experimentally induced metamorphosis of the skin, the cercariae of *Glythelmins* were able to penetrate and become encysted as in the skin of normally metamorphosed frogs.

## REFERENCES

- BYRD, E. E. 1935 Life history studies of Reniferinae (Trematoda, Digenea) parasitic in Reptilia of the New Orleans area. Trans. Amer. Micros. Soc. 54: 192-225.
- HELFF, O. M. 1931 Studies on amphibian metamorphosis. 9. Integumentary specificity and dermal plicae formation in the anuran, *Rana pipiens*. Biol. Bull. 60: 11-22.
- LEIGH, W. H. 1937 The life cycle of a trematode of frogs. Sci. 86: 423.
- 1937a The life cycle of a trematode of frogs. Jour. Parasitol. 23: 563.
- (in press) Experimental studies on the life cycle of *Glythelmins quieta* (Stafford, 1900), a trematode of frogs. Amer. Midl. Nat.
- MCCOY, O. R. 1928 Life history studies on trematodes from Missouri. Jour. Parasitol. 14: 207-228.
- McMULLEN, D. B. 1935 The life histories and classification of two Allocreadiid-like plagi-orchids from fish, *Macroderoides typicum* (Winfield) and *Alloglossidium corti* (Lamont). Ibid. 21: 369-380.
- 1937 The life histories of three trematodes parasitic in birds and mammals, belonging to the genus *Plagiorchis*. Ibid. 23: 235-243.
- NOBLE, G. K. 1931 The Biology of the Amphibia. McGraw-Hill, N. Y.
- RANKIN, J. S. 1944 A review of the trematode genus *Glythelmins* Stafford, 1905, with an account of the life cycle of *G. quieta* (Stafford, 1900) Stafford, 1905. Trans. Amer. Micros. Soc. 63: 30-49.
- TALBOT, S. B. 1933 Life history studies on trematodes of the subfamily Reniferinae. Parasitol. 25: 518-545.
- WILDER, I. W. 1925 The morphology of amphibian metamorphosis. Smith College 50th Anniv. Publ. 1-161.



A NEW TREMATODE, *NEORENIFER CROTALI*, FROM  
THE RATTLESNAKE<sup>1</sup>

J. TEAGUE SELF

The specimens on which this paper is based were collected in March, 1942, from a specimen of *Crotalus atrox* Baird and Girard 1853, by Frank B. McMurry, wildlife technician, in the Wichita Mountains Wildlife Refuge.<sup>2</sup> Ninety specimens were taken from the lower lung, mouth and esophagus of the host. The latter was killed by drowning and since most of the flukes were found imbedded in the tissue of the lower lung it is assumed that those found in the mouth and esophagus were washed there, although this must remain a point in question. They were fixed in hot 70% alcohol and the ten *toto* mounts used in this study were stained in either Semichon's aceto-carmin or Mayer's paracarmine. One specimen was sectioned serially for study of the excretory system.

The specimens belong to the genus *Neoreniker* Byrd and Denton, 1938, and apparently to a new species. Appreciation is due Mr. McMurry for furnishing

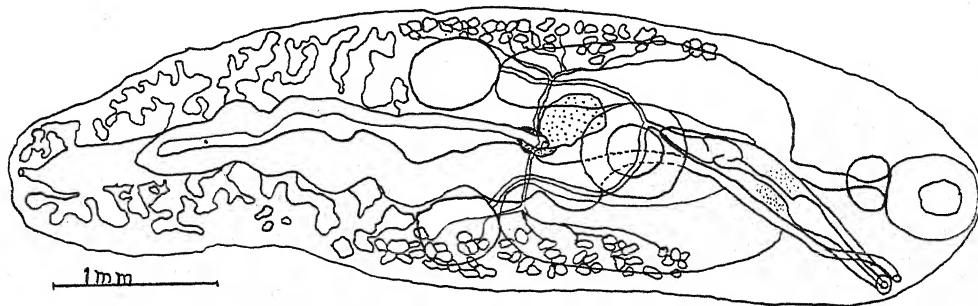


FIG. 1. Drawn from projection of the type specimen.

the specimens, five of which are to be deposited in the United States National Museum.

*Neoreniker crotali* sp. nov.

*Host:* *Crotalus atrox atrox* Baird and Girard.

*Location:* Lower lung.

*Description:* Cuticular spines absent. Tube-like integumentary glands numerous in anterior half of body. Body ovate, slightly more pointed at the posterior end and widest in the region of the testes. Average length 4.89 mm (3.50–6.22), average width 1.31 mm (0.91–1.70). Ratio of length to width 3.7:1. Diameter of acetabulum 0.47 mm and of oral sucker 0.42 mm with a ratio of 1.2:1. Length of esophagus 0.37 mm. Ratio of body length to length of esophagus 15.2:1. Length of cirrus pouch 1.37 mm. Ratio of body length to that of cirrus pouch 3.5:1. The cirrus pouch completely incloses the seminal vesicle and extends dorsal to the right anterior part of the acetabulum. The seminal vesicle is roughly S-shaped or slightly spiraled and is joined to the cirrus organ by a slender duct. The prostate is indistinct. The genital pore opens on the left margin and at the level of or slightly anterior to the level of the oral sucker-pharynx juncture.

The ovary is dextrosubmedian and partially overlaps the acetabulum. The diameter of the ovary is 0.24 mm. The shell gland is poorly defined and consists of a glandular tissue around the proximal end of the uterus. The eggs are oval in shape, averaging 0.04 mm in length and 0.02 mm in thickness. The testes are opposite, and slightly posterior to the midregion of the body.

Received for publication, April 5, 1945.

<sup>1</sup> Contribution from the Zoological Laboratory, University of Oklahoma.

<sup>2</sup> Now Acting Refuge Manager, Havasu Lake National Wildlife Refuge.

The diameters of the testes, 0.36 mm. The testes vary from a spherical to slightly ovate, but not lobed, condition. The vasa efferentia join just before entering the cirrus pouch. The vitellaria are marginal in the central third of the body and are not divided into anterior and posterior groups.

The excretory vesicle extends from the slightly subterminal excretory pore to the level of the testes where it connects with two large lateral tubules. It is profusely lobed, the lateral lobes being highly convoluted.

#### DISCUSSION

The new species is more closely related to *N. septicus* (McCallum, 1921) and *N. serpentis* Schmidt and Hubbard, 1940, than to any others, accepting the opinion of Byrd and Denton (1938) that *R. ophiboli* McCallum, 1921, is a synonym of *N. septicus*. It differs from both *N. septicus* and *N. serpentis* in the host and the position which it occupies in the host. It differs from *N. septicus* in having a larger pharynx in relation to the size of the oral sucker (ratio of 1.7:1 as compared to 2.5:1); in having a shorter esophagus, (ratio to body length of 1:15.2 as compared to 1:9.3) and in having the genital pore marginal and much more anteriorly located. It differs from *N. serpentis* in having a lobed excretory vesicle, in having a marginal genital pore, a longer esophagus, and a less distinct shell gland.

In addition to *N. septicus*, and *N. serpentis* fourteen other species belonging to this genus are known to the author. Information on *N. grandispinus* was taken from Manter (1943) and on the others which follow from Byrd and Denton (1938). The absence of cuticular spines distinguishes the new species from *N. wardi* (Byrd, 1936), *N. kansensis* (Crow, 1913), *N. formosum* (Nicoll, 1911), *N. elongatus* (Pratt, 1903), *N. zschokkei* (Volz, 1899), *N. validus* (Nicoll, 1911), *N. sauro-mates* (Poirier, 1885), *N. georgianus* Byrd and Denton, 1938, *N. heterodontis* Byrd and Denton, 1938, *N. glandularis* Byrd and Denton, 1938, and *N. grandispinus* (Caballero, 1938). It is distinguished from *N. acetabularis* (Crow, 1913) and *N. aniarum* (Leidy, 1890) by the continuous arrangement of the vitellaria and from *N. orula* (Talbot, 1934) by the sinistral position of the genital pore.

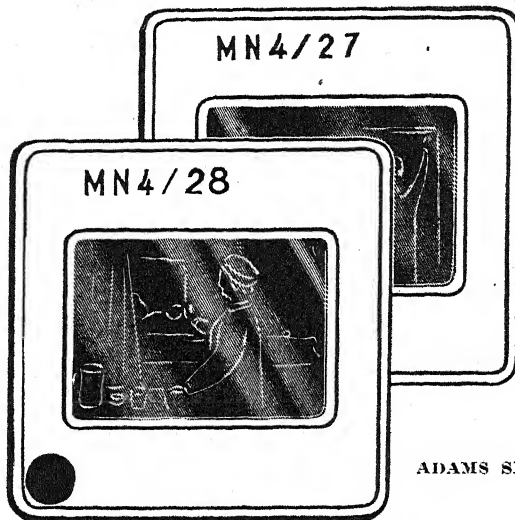
Certain striking similarities to both *N. glandularis* and *N. grandispinus* are apparent but it is necessary to keep it distinct from these species because of the absence of cuticular spines.

Schmidt and Hubbard (1940) have pointed out that *N. serpentis* may be a synonym of *N. septicus*. It seems definitely to be distinct, however, because it lacks the lobed excretory vesicle apparent in *N. septicus*. The species described here seems more likely to be a synonym of *N. septicus* although the description of the latter by McCallum makes comparison of other specimens with it difficult. The marked differences apparent in the length of the esophagus, level of the genital pore, size of the pharynx in relation to that of the oral sucker and in the position in the host as well as their being in widely separated host species seem to justify the establishment of a new species.

#### REFERENCES

- BYRD, ELON E. AND DENTON, J. FRED 1938 New trematodes of the subfamily Reniferinae with a discussion of the systematics of the genera and species assigned to the subfamily group. *J. Parasitol.* 24: 379-401.
- MCCALLUM, G. A. 1921 Studies in helminthology. *Zoopathologica*, N. Y. Zool. Soc., 1: 137-284.
- MANTER, HAROLD W. 1943 One species of trematode, *Neoreniker grandispinus* (Caballero, 1938), attacked by another, *Mesocercaria marciana* (LaRue, 1917). *J. Parasitol.* 29: 387-392.
- SCHMIDT, FRED L. AND HUBBARD, W. EUGENE 1940 A new trematode, *Neoreniker serpentis*, from the water moccasin. *Amer. Mid. Nat.* 23: 729-730.

# AIDS for VISUAL EDUCATION



## MEDICHROMES

2×2" Kodachrome lantern slides in the medical and biological sciences, as described in our Catalogs Nos. 103 and 151. Write for supplementary listings of slides in your own field.

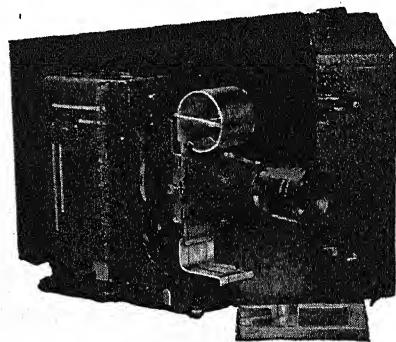
ADAMS SLIDE BINDER

## PROJECTORS

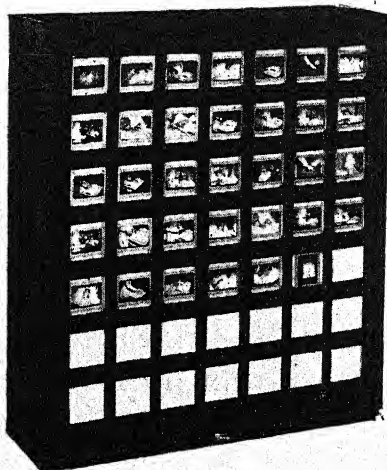
Model AAA (accommodates 2×2" slides and 35 mm. single and double frame strip film). Complete with 300-watt lamp, 5" anastigmat lens, semi-automatic vertical slide changer, leatherette carrying case. A-1500 ..... \$65.00

With Rewind Take-Up

A-1513 ..... \$5.00 additional



MODEL AAA A-1500



KODACHROME VIEW BOX

Also other SVE Projectors, Spencer Delineascopes and Golde Projectors. Write for descriptive literature.

Also Kodachrome View Boxes, Slide Binders, Hand Viewers, Screens, Kodachrome Slide Boxes and Kodachrome Slide Files.

Write for Form 371JP

## CLAY-ADAMS CO. INC.

44 EAST 23rd STREET, NEW YORK 10, N. Y.



# The Journal of Parasitology

Volume 31

AUGUST, 1945

Number 4

## STUDIES IN CESTODE CYTOLOGY\*

ARTHUR W. JONES

Miller School of Biology, University of Virginia

### I. INTRODUCTION

In opposition to the chromosome theory of heredity, or at least in question of it, stand most of the few existing reports on the cytology of cestodes. In a series of papers Child (1904 et seq.) attempted to show the frequent occurrence of amitosis in cestode nuclei. He described and figured stages of amitosis, showing nuclei partially constricted while in an apparent resting stage. He figured also, as illustrative of the result of amitosis, nuclei of the same size closely adjacent to each other. Thus, by the method of finding cells which represented all stages in the hypothetical process, Child claimed the existence of the process. He admitted the existence of regular mitosis in cestodes, but denied that it could be demonstrated frequently enough to support the view that it was common. Richards (1911) called attention to the assumption inherent in Child's evidence, denied that Child's "stages of amitosis" were necessarily anything but selected chance deformations and orientations of resting nuclei, and, finally, showed the regular occurrence of mitosis and meiosis in gametogenesis and cleavage of the same cestode, *Moniezia expansa* (Rudolphi), upon which Child had based his hypothesis. Child (1911) responded by publishing his own interpretations of Richards' slides, which, he said, actually supported his own view. Harman (1913) described gametogenesis in another cestode, *Taenia pisiformis* Bloch, supporting Richards' conclusions that cestode nuclei, at least in gametogenesis, divide mitotically. Neither Richards nor Harman presented any evidence of the continuity of the chromosome complement throughout growth; they failed to give the number or morphology of the chromosomes of their respective species. Thus their arguments were open to the objection that amitosis might have functioned in the somatic divisions preceding differentiation of the gonads. Both Harman and Richards figure variation in numbers and kinds of chromosomes (which would, of course, result from the crude process of amitosis). Young (1910) described the somatic nuclei of twelve larval or mature cestodes, concluding that, in all of these worms, nuclei normally develop from cytoplasmic granules. Young (1912) stated (p. 399): "In a total of 33,930 nuclei counted in the Cestode soma, mainly in actively growing regions, I have observed only 50 mitoses. . . . Many cases of probable amitosis occur." Young (1923) described gametogenesis in cestodes, indicating that in his opinion the division and maturation of even the repro-

Received for publication, September 14, 1944.

\* Contribution from the Miller School of Biology, University of Virginia. The author gratefully acknowledges the help of Dr. B. D. Reynolds, under whose direction the work was done, and of Dr. Ladley Husted, who gave valuable advice on the cytological problems.



ductive nuclei is extremely irregular. Young here (p. 435) advanced the "theory that mitosis is degenerating" in the cestodes. Since this paper only one work has been published on cestode cytology, Motomura's (1929) report of gametogenesis in the paedogenetic cestode *Archigetes appendiculatus* Ratzel. This work gives the chromosome number, describes a conventional meiosis and mitosis, and contains no suggestion that *Archigetes* differs, in its method of nuclear division, from other higher organisms. The fact that *Archigetes* is a rather anomalous form, being a monozoic or "unsegmented" cestode parasite of an invertebrate, makes Motomura's contribution not especially pertinent to the discussion of cestode cytology in general. The succeeding studies are an attempt to apply modern cytological methods to the problem.

## II. TECHNICAL METHODS

Prior workers' methods having yielded, on the whole, unsatisfactory results, it was necessary to test these methods, as well as try other methods in common use by cytologists. Several strobilae of the same species, from the same host individual, were considered, for trial purposes, to be practically identical. The effects of fixation of these cestodes severally in various fixing solutions were studied and compared, and the fixative which proved best by this comparison was then used in other tests. Where only one strobila was recovered from a host, it was cut up, in saline solution, into several parts, and the parts fixed in different solutions. Both methods gave useful results. See Table 1.

### *Smear Methods*

Painter's (1939) technique, using acetocarmine on Carnoy-fixed material torn up with steel needles and pressed under a coverglass, with heat, was successful in making tentative chromosome-number determinations. It is not suitable for critical work. The Feulgen reagent, used with Heitz's "Quetschmethode" as suggested by Bhaduri and Semmens (1942), gives sharper stain and better final fixation after Carnoy than does acetocarmine. Fixed material, in water, is mordanted three hours in 1% chromic acid. Rinsed, hydrolyzed in normal HCl at 40° C. 60-80 minutes, the worms are again rinsed, then stained in the Feulgen reagent. Then the worms are cleared in 50% glacial acetic; selected regions are removed, macerated, and pressed under a cover glass. If properly made, as seen under oil, the smear may be made permanent by passage very slowly through 1:1 glacial acetic ethanol, the cover removed, dehydrated, cleared, and re-mounted. Counterstaining for nucleoli (see Bhaduri and Semmens, 1942) is desirable.

### *Sections*

Fixation for sectioning was done by the following reagents. Carnoy's, either 3:1 alcohol-acetic, or 6:3:1 alcohol-acetic-chloroform, is a good general fixative, to be followed preferably by the Feulgen reagent or iron haematoxylin. (It is also good for total mount preparations, where identification depends upon those.) Navashin's chrom-acetic-formalin, as used by Husted and Burch (1943) on chromosomes of snails, was not successful, tending to clump the chromosomes. Flemming's osmic acid fixatives, both the "medium" solution and LaCour's 2BE (see LaCour, 1937), gave the best fixation of chromosomes. Of these, the former has little penetrating power, the latter is best for cleavage stages in embryos. Fixatives containing sodium diuranate (Bhaduri and Semmens, 1942), tried upon *Hymenolepis fraterna*



only, gave excellent results, justifying further use. For other fixatives tried, see Table 1.

Dehydration through an ethanol series into chloroform, with slow embedding in paraffin, resulted in successful preparations. See LaCour (1931).

Sections were cut thick enough so that several layers of whole nuclei could be in one section. For cestodes, 14  $\mu$ , in general, is satisfactory. Thinner sections may contain valuable mitotic or meiotic figures that have been ruined. Reconstruction of chromosome complements (see Richards, 1911; Pennypacker, 1940; Rees, 1939) is cytologically untrustworthy, and may be avoided by thick sections.

Sections may be stained with crystal violet (Newton and Darlington, 1929), the Feulgen reagent, or iron haematoxylin. The first is not permanent, nor does it follow Carnoy's successfully. The third is more difficult than the others, because the differentiation cannot be easily observed and controlled. The second, therefore, seems the best general stain for cestode chromosomes, since, once the technique is mastered, it is applicable to most material.<sup>1</sup>

TABLE 1. Summary of fixation trials

	No. trials	Satisfactory	Unsatisfactory
Carnoy (6:3:1) or (3:1) .....	15	14	1
Medium Flemming .....	15	10	5
Strong Flemming .....	5	2	3
LaCour 2BE .....	9	6	3
Bouin .....	1	0	1
Allen B15 .....	2	0	2
Webber's Navashin* .....	1	1	0
San Felice Navashin .....	3	2	1
Sodium diuranate (2 formulas) .....	2	2	0
Totals	53	37	16

\* Tissue fixation was so poor as to make identification impossible.

### III. CHROMOSOMES OF THE FAMILY HYMENOLEPIDIDAE

#### *The 10-diploid Hymenolepididae*

1. *Protogynella blarinae* Jones, 1943 (host, *Blarina brevicauda* Say, 1823), may have in its neck region plentiful mitoses, which fix well with Medium Flemming. The chromosomes (Fig. 2) may be easily classified as five pairs, supposedly homologous. The idiogram, showing one large pair, two middle-sized pairs, and two smaller pairs, was drawn from a study of ten clear mitoses.

The heteromorphic, or "tailed" chromosome shown in the third pair of the figure is characteristic of the members of the species studied. It may be considered, perhaps, to consist of a portion normally condensed at metaphase, together with another portion only partially condensed.<sup>2</sup>

<sup>1</sup> The time of hydrolysis, at 40° C., may usually be 60 minutes. Some evidence of specificity for optimum period of hydrolysis appeared during tests upon 16 cestodes, indicating that an individual optimum might be determinable for an individual species being investigated.

<sup>2</sup> Such a condition, which seems the simplest interpretation in line with described phenomena, is found in the Y chromosome of certain higher organisms, e.g., *Rattus norvegicus* and certain other mammals. (See Koller and Darlington, 1934.) Therefore, the heteromorphic chromosome suggests by analogy a sex-determining mechanism. The cestode in which it is found was described (see Jones, 1943a) as strongly protogynous, a condition, rare in the cestodes, which may be a step toward the dioecious condition found in cestodes of the genus *Diococystus* Fuhrmann. If protogyny were dependent upon factors in the heteropycnotic arm of the dimorphic chromosome, a sex-determining mechanism would indeed be present. Two kinds of gametes would be produced, and a progeny segregating for protogyny and a hypothetical protandry could arise. The writer believes that life history and breeding experiments on these cestodes should be undertaken.

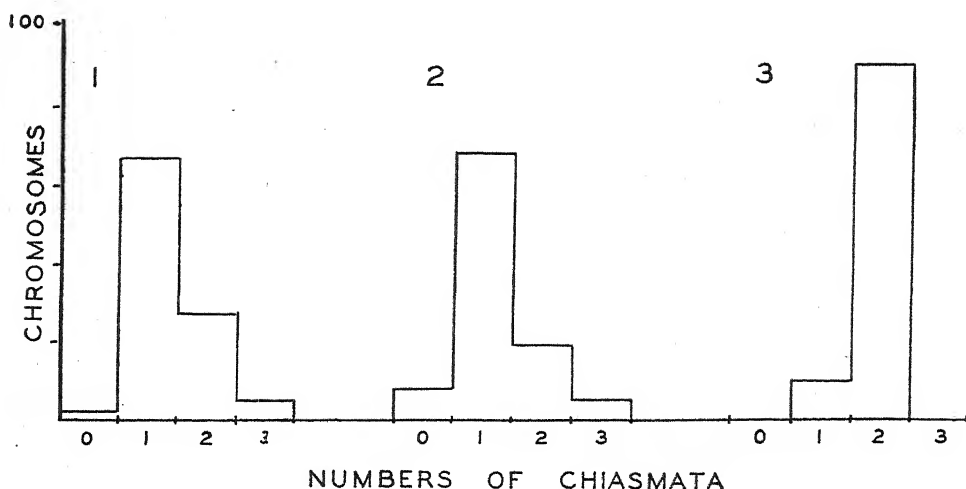


FIG. 1. Chiasma frequencies in three species of cestodes, *Hymenolepis anthocephalus* (1), *Diorchis reynoldsi* (2), and *Hymenolepis serpentulus* "sturni" (3). Chiasmata were counted at diplotene or early diakinesis in each case. Numbers of chromosomes partaking in the formation of no, one, two, and three (the maximum number observed in any bivalent) chiasmata are plotted against the numbers of chiasmata per bivalent.

2. *Diorchis reynoldsi* Jones, 1944, is a co-parasite with the preceding cestode, *Protogynella blarinae*. Its chromosomes (Fig. 3) are somewhat larger than those of *P. blarinae*, but, like them, consist of five pairs. The largest pair has submedian centromeres. The next pair, nearly as long as the first, has subterminal centromeres, as do the three smaller pairs. The chromosomes resemble those of *Hymenolepis fraterna* (Fig. 5) more than they do those of the other members of the family.

In a few preparations diplotene configurations were found, and the number of chiasmata could be counted for each bivalent (Fig. 1). *Diorchis reynoldsi* was found to have a chiasma frequency of 1.23 per bivalent, based on diplotene data. Table 2 compares the chiasma frequencies of five cestodes, including *D. reynoldsi*.

In *D. reynoldsi* meiotic figures showing more than the five bivalents expected on the basis of the mitotic number could be explained as the results of failure of chiasmata. Meiotic nuclei which had extra "bivalents" usually had two distinctly smaller ones (oriented on the spindle, however) plus four large ones; while in plates of five bivalents the two small ones were replaced by a larger one of approximately the same size as the other four. The extra "bivalents" were thus seen to be univalents. These become orientated upon the spindle (unlike most univalents in other organisms), but do not divide at the first division of meiosis. Secondary

TABLE 2.—Chiasma frequencies in five hymenolepidid cestodes

Species	No. bivalents	No. chiasmata	Chiasmata per bivalent
<i>Hymenolepis anthocephalus</i> .....	419	624	1.49
<i>Diorchis reynoldsi</i> .....	73	90	1.23
<i>H. serpentulus sturni</i> .....	642	644	1.01*
<i>H. s. turdi</i> .....	438	500	1.07
<i>H. fraterna</i> .....	2290	2146	0.94*

\* These determinations are minimum frequencies based on study of meiotic metaphases, on the assumption that at least one chiasma must be found in each bivalent.

spermatocytes were found containing six or four chromosomes as well as the usual five, indicating that the univalents pass at random to the poles.

Five different strobilae of *D. reynoldsi* were studied and compared to see how regular was the occurrence of univalents. Table 3 shows that while the average proportion of cells showing complete pairing to those showing two or more univalents is 3:1; the strobilae vary considerably around this figure. A statistical analysis, using the Probable Error of the ratios and of the mean ratio, shows that most of the variations among these values can be explained as due to sampling error (i.e., the differences are usually less than three times their P.E.; Snyder, 1940, p. 336). It is probable, therefore, that the amount of univalent occurrence in *D. reynoldsi* is a chance phenomenon, connected with the random formation of chiasmata, and is not a constant character for any worm as against another. Univalent frequencies in the individual strobilae then appear as reflections of varying chiasma frequencies. (The latter, it is believed by some, may be subject to environmental or other influences not discernible or classifiable in this material. See Darlington, 1937, page

TABLE 3.—Univalent frequencies in five strobilae of *Diorchis reynoldsi*

Strobila	Numbers of nuclei in		Ratio Class I Class II $\pm$ P.E.*	Significance of difference		
	Class I + II + 2 I	Class II 5 II		Strobila	Difference $\pm$ P.E.†	Difference P.E.
1	3	12	0.25 $\pm$ 0.097	3-1	0.35 $\pm$ 0.11	3.2
2	0	4		1-1	0.09 $\pm$ 0.09	1.0
3	6	10	0.60 $\pm$ 0.083	5-1	0.20 $\pm$ 0.09	2.2
4	4	25	0.16 $\pm$ 0.046	3-4	0.34 $\pm$ 0.10	3.4
5	19	48	0.45 $\pm$ 0.046	3-5	0.15 $\pm$ 0.10	1.5
				5-4	0.29 $\pm$ 0.06	4.8
T	32	93	0.344 $\pm$ 0.0286	T-1	0.09 $\pm$ 0.08	1.1
				T-4	0.18 $\pm$ 0.05	3.6
				3-T	0.24 $\pm$ 0.09	2.7
				5-T	0.11 $\pm$ 0.05	2.2

\* P.E. of a proportion,  $p = \pm 0.6745 \sqrt{\frac{p(1-p)}{n}}$ .

† P.E. of a difference  $= \pm 1/\sqrt{(P.E._1)^2 + (P.E._2)^2}$ .

168.) The univalent frequency of strobila 4, as seen in table 3, perhaps differs significantly from that of the other strobilae, except strobila 1, as well as from the total frequency. Since all strobilae here included came from the same host individual, environmental conditions may be assumed the same for all. Whether or not the divergent univalent frequency of strobila 4 is due to genotypic control is impossible to say. This matter will be taken up in the discussion of *Hymenolepis fraterna*.

3. *Hymenolepis fraterna* Stiles, 1906, obtained from the rat, has ten chromosomes in the mitotic complement (Fig. 5). One pair, the largest, has median centromeres. The rest have subterminal centromeres. Besides the pair of large chromosomes, there are two pairs of middle-sized, and two pairs of small. There is a marked similarity, noted above, between the chromosomes of *H. fraterna* and those of *Diorchis reynoldsi*.

The chiasma frequency of *H. fraterna* could be found only as a function of the univalent frequency (Table 4). Diplotene stages were not well preserved by any of the fixatives used. While it is recognized that the relation between low chiasma frequency and univalent occurrence may not be as simple as a mathematical function, yet one may make the assumption for limited purposes of comparison. (Table 2

illustrates simply the differences and similarities of certain closely related cestodes in terms of a character, chiasma frequency, which may, or may not, have actual taxonomic value. The compilation of many more cytological tables, like Tables 2, 3 and 4 herein, might, indeed, establish such a value in chiasma frequencies.) The chiasma frequency of *H. fraterna*, on the assumption of at least one chiasma per bivalent to give complete pairing, is ca. 0.95 chiasma per bivalent. This value is derived from study of three strobilae (460 cells) from a single host.

The univalent frequencies in these three strobilae appear to vary over a considerable range (Table 4). By computing the Probable Error for proportions, and deriving the P.E. of differences (see Snyder, 1940: 336, 337), it was determined that the differences between most of the ratios are probably not due to sampling error alone. It can therefore be said that univalent frequencies do vary, both as between different strobilae, and as between different sexes in the observed specimens of *Hymenolepis fraterna*.

TABLE 4.—Univalent frequencies in spermatocytes and oöcytes of three strobilae of *Hymenolepis fraterna*

Strobila and sex of tissue	Numbers of nuclei in		Ratio Class I Class II $\pm$ P.E.*	Significance of difference		
	Class I 4 II + 2 I	Class II 5 II		Tissues and strobilae	Difference $\pm$ P.E.	Difference P.E.
$\sigma^2$ a	37	66	0.56 $\pm$ 0.033	$\sigma^2$ a - $\sigma^2$ b	0.16 $\pm$ 0.046	3.5
$\sigma^2$ b	30	75	0.40 $\pm$ 0.032	$\sigma^2$ c - $\sigma^2$ a	0.26 $\pm$ 0.053	4.9
$\sigma^2$ c	18	22	0.82 $\pm$ 0.041	$\sigma^2$ c - $\sigma^2$ b	0.42 $\pm$ 0.054	7.8
Total	85	163	0.520 $\pm$ 0.0233	$\sigma^2$ a - $\sigma^2$ b	0.22 $\pm$ 0.046	4.8
$\phi$ a	No data	76	0.34 $\pm$ 0.032	$\phi$ a - $\phi$ b	0.27 $\pm$ 0.046	5.9
$\phi$ b	26	79	0.31 $\pm$ 0.031	$\phi$ b - $\phi$ c	0.06 $\pm$ 0.045	1.3
$\phi$ c	23	79	0.29 $\pm$ 0.031	$\phi$ b - $\phi$ c	0.11 $\pm$ 0.045	2.4
Total	49	155	0.316 $\pm$ 0.0214	$\phi$ c - $\phi$ b	0.48 $\pm$ 0.045	10.0
$\sigma^2$ $\phi$ b	56	151	0.370 $\pm$ 0.0212	$\phi$ c - $\phi$ b	0.53 $\pm$ 0.052	10.0
$\phi$ c	31	101	0.308 $\pm$ 0.0272	$\phi$ b - $\phi$ c	0.05 $\pm$ 0.045	1.1
				$\sigma^2$ T - $\phi$ T	0.204 $\pm$ 0.030	6.8
				$\sigma^2$ $\phi$ b - $\sigma^2$ $\phi$ c	0.062 $\pm$ 0.0313	1.9

\* See footnote to Table 3.

Univalent occurrence affected zygotic chromosome number in this cestode. The first cleavage of at least two out of eight eggs (selected only for clearness of division figure) showed a diploid complement of twelve, two more than the somatic complement of the parent. It may be supposed that two gametes, each with six chromosomes, fused to form such zygotes. Such gametes might frequently arise in strobilae whose meiotic divisions show a high univalent frequency, considering the known tendency of univalents in other material to segregate irregularly to the daughter nuclei of the first division. This possibility was suggested as an explanation for observed chromosome number variation in a turbellarian; Jones, 1943b.) A broad survey of chromosome number in *Hymenolepis fraterna* might reveal the survival as adults of the 12-diploid zygotes.<sup>3</sup>

<sup>3</sup> The problem of variation in cestodes has received both taxonomic (e.g., Venard, 1938) and physiological (e.g., Larsh, 1943 et seq.) attention. The conclusions reached in regard to the problem cannot be considered finally valid, however, until some attention be given the questions of genetic uniformity and stability in the species or strains considered. The author's finding of frequent zygotic variation in the early embryos within the uteri of a single strobila of *Hymenolepis fraterna* (or *H. nana* var. *fraterna*, the form with which Larsh, 1943 et seq., experimented) suggests the possibility of heterozygosity existing in a rigidly selected "strain."

4. *Diorchis ralli* Jones, 1944, from the King Rail, has a diploid complement of 10. Unlike the preceding worms, it has (Fig. 4) two pairs with median centromeres, one large, the other, middle-sized. There is one intermediate pair with subterminal centromeres, and there are two small pairs in which the centromeric constriction is not evident. In meiosis, good evidence was found for the occurrence of univalents. Metaphase I of meiosis is shown in the second figure (Fig. 4), with univalent chromosomes near the poles of the spindle, and four bivalents orientated at the equator.

#### *The 12-diploid Hymenolepididae*

1. *Hymenolepis anthocephalus* van Gundy, 1936, has 12 chromosomes, consisting of two large pairs (one with submedian centromeres and one with subterminal centromeres), and of four small pairs. The chromosomes of this cestode resemble those of *Diorchis reynoldsi* (Fig. 3) more than any other of the group.

*H. anthocephalus* presented further opportunity for chiasma study, its meiotic prophases being exceptionally well preserved. About 420 bivalents at diplotene were studied and the number of chiasmata counted. As appears from Fig. 1, the curve of distribution of chiasmata is quite similar, in *H. anthocephalus*, to the curve for *D. reynoldsi*, whose chromosomes show similarity to those of *H. anthocephalus*. The relative absence of univalents, with the corresponding increase in the number of bivalents with more than one chiasma, reflects the higher frequency *per* bivalent found in *H. anthocephalus* (see Table 2). On the basis of meiotic pairing, then, *H. anthocephalus* may be considered a more stable organism than either *D. reynoldsi* or *H. fraterna*.

#### Chromosome aberrations in *Hymenolepis anthocephalus*

In one strobila (which morphologically resembled other strobilae of *Hymenolepis anthocephalus*) a radical irregularity in chromosome behavior occurred. This was the presence of ring chromosomes in the somatic mitoses (with the apparent consequences of this aberration) traceable all the way from the proliferating neck to the developing gametes.

In the somatic mitoses of the aberrant cestode are frequent ring and dicentric chromosomes at metaphase, and chromosome bridges at anaphase and telophase (see Fig. 10). Chromosome counts of eight, nine, ten, and occasionally more, can be made from somatic metaphases. The number twelve, normal for the species, is rare. The lengths of the chromosomes, and the sizes of rings, vary noticeably in neighboring nuclei. At anaphase, bridges are strikingly frequent; of one hundred anaphases or early telophases picked at random, about sixty had unmistakable bridges. Others showed evidence of breakage of an early bridge. Five or six anaphases had two bridges, involving, therefore, two or more chromosomes. The "normal," or bridgeless, anaphases were, therefore, exceptional.

In meiotic prophases rather complex configurations, capable of several interpretations, appear (see Fig. 10, J). According to their apparent structure and

---

Physiological or morphological variation in the strain should not then be attributed solely to environmental differences supplied by different experimental hosts, but may be attributed also to the selective effect of such environments. Continued breeding of the selected strains in the different hosts, coupled with cytological examination of the cestodes, could eliminate the uncertainty inherent in Larsh's (1943 et seq.) and Venard's (1938) experiments.



## MITOSIS AND MEIOSIS

## IDIOGRAM



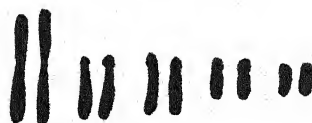
2



3



4



5

FIGS. 2-5. Description: All figures ca. 4300×

FIG. 2. *Protogynella blarinae*: Mitosis, showing ten chromosomes, including the heteromorphic one; meiosis, metaphase I, showing five bivalents; and an idiogram\* for the species, derived from estimated measurements of ten metaphase plates.

\* The word idiogram is used in this paper not in its technical sense, but in the sense of a convenient arrangement of depicted chromosomes, for the purpose of illustrating chromosome morphology.

orientation, they are multivalent associations of chromosomes. While they are too small to show chiasmata in detail, they may have what looks like a triple chiasma involving close end-to-end association of three homologues in the pattern known as "ring-and-rod trivalent" in the well known *Datura* configuration, or they may be more complex, like the "quadrivalent" drawn, diagrammed, and photographically reproduced in Fig. 10, G, which seems to involve four chromosomes. In meiotic metaphase I, the ring-rod configurations persist, and assume a conventional appearance, except that the ring constituent may be disproportionate in size to the rod. More than one ring-rod may be present in one nucleus. Unfortunately, the inconstancy of chromosome number in the premeiotic figures makes it impossible, by a count of the other chromosomes in the nucleus, to ascertain how many chromosomes actually take part in those associations.

The aberrations reported above may be accounted for by McClintock's (1932 et seq.) clear description of the way in which ring chromosomes behave. In maize, ring chromosomes at mitosis may divide in one plane, in two, or in several. Thus, at mitotic anaphase, identical freely separating rings may result from division in one plane, a "double-sized ring chromosome" results from division along two planes, and interlocking rings result from division along three planes. Both double-sized rings and interlocks form chromosome bridges at anaphase, which must break for separate nuclei to form. As McClintock showed in maize, the broken ends reunite, or unite with other broken ends, to form either new rings or dicentric chromosomes in the daughter nuclei. (Dicentric chromosomes may also arise from the same conditions that produce rings.)

The ring-bridge cycle produces great changes in daughter nuclei. Small rings may not be able to separate, if interlocking, and may become thus duplicate in one daughter nucleus and absent in the other. This fact rather easily explains the number variations in somatic chromosomes mentioned above. The fact that most of the nuclei of the aberrant cestode have less than the twelve chromosomes normal for the species suggests the frequent loss in the cytoplasm (as described by McClintock, 1938b) of small ring chromosomes. Mitotic variation in chromosome length (such as that found in the cestode) can be explained on the assumption of irregular breakage of double-sized rings, such rings having grown by continuation of the ring-bridge cycle. The limit of growth for a ring is probably reached when broken ends are too far apart (chromosome has become too large, or the breaks occur too far from each other) for them to reunite. The limit of shrinkage is reached when the interlocking rings are too small fully to separate at anaphase, so that complete loss or inclusion in only one daughter nucleus occurs.

Meiotic configurations are affected, whether the incidence of ring chromosomes is low, as in untreated maize, or high, as in X-rayed maize or the aberrant cestode. From breakage at different places along a ring chromosome at different divisions, the order and number of genes may be shifted, so that the ring chromosome becomes structurally different from its rod homologue. Where two rings exist in a single

FIG. 3. *Diorchis reynoldsi*: Mitosis, meiosis (I and II), and idiogram derived from several clear mitoses. Four bivalents and two univalents are shown in meiosis I.

FIG. 4. *Diorchis ralli*: Mitosis, meiosis (I and II), and idiogram. Four bivalents and two univalents shown in meiosis I.

FIG. 5. *Hymenolepis fraterna*: Mitosis, meiosis (I and II), and idiogram. Five bivalents shown at meiosis I. Note separate arms of chromosomes at metaphase II.

## MITOSIS AND MEIOSIS

## IDIOGRAM



6



7



8



9

FIGS. 6-9. Description: All figures ca. 4250 $\times$ .

FIG. 6. *Aploparaksis* sp.: Mitosis (a late prophase), meiosis I (diakinesis), meiosis II, and idiogram. Note heteromorphic chromosome shown in mitosis and idiogram.

FIG. 7. *Hymenolepis serpentulus sturni*: Mitosis, meiosis I (diakinesis), meiosis II, and idiogram. Note especially the appearance of duplicated pairs of chromosomes; is the base haploid set 3, ---- two submedians and a subterminal? In this tentative subspecies a few "quadrivalents" were seen.

FIG. 8. *Hymenolepis serpentulus turdi*: Mitosis, meiosis I (diakinesis), meiosis II, and idiogram. Note differences in size and morphology between these chromosomes and those of the other tentative subspecies, Fig. 7.

FIG. 9. *Hymenolepis anthocephalus*: Mitosis, meiosis I (metaphase), meiosis II, and idiogram. Compare this with Fig. 3, noting similarity in morphology between these parasites of the same host.

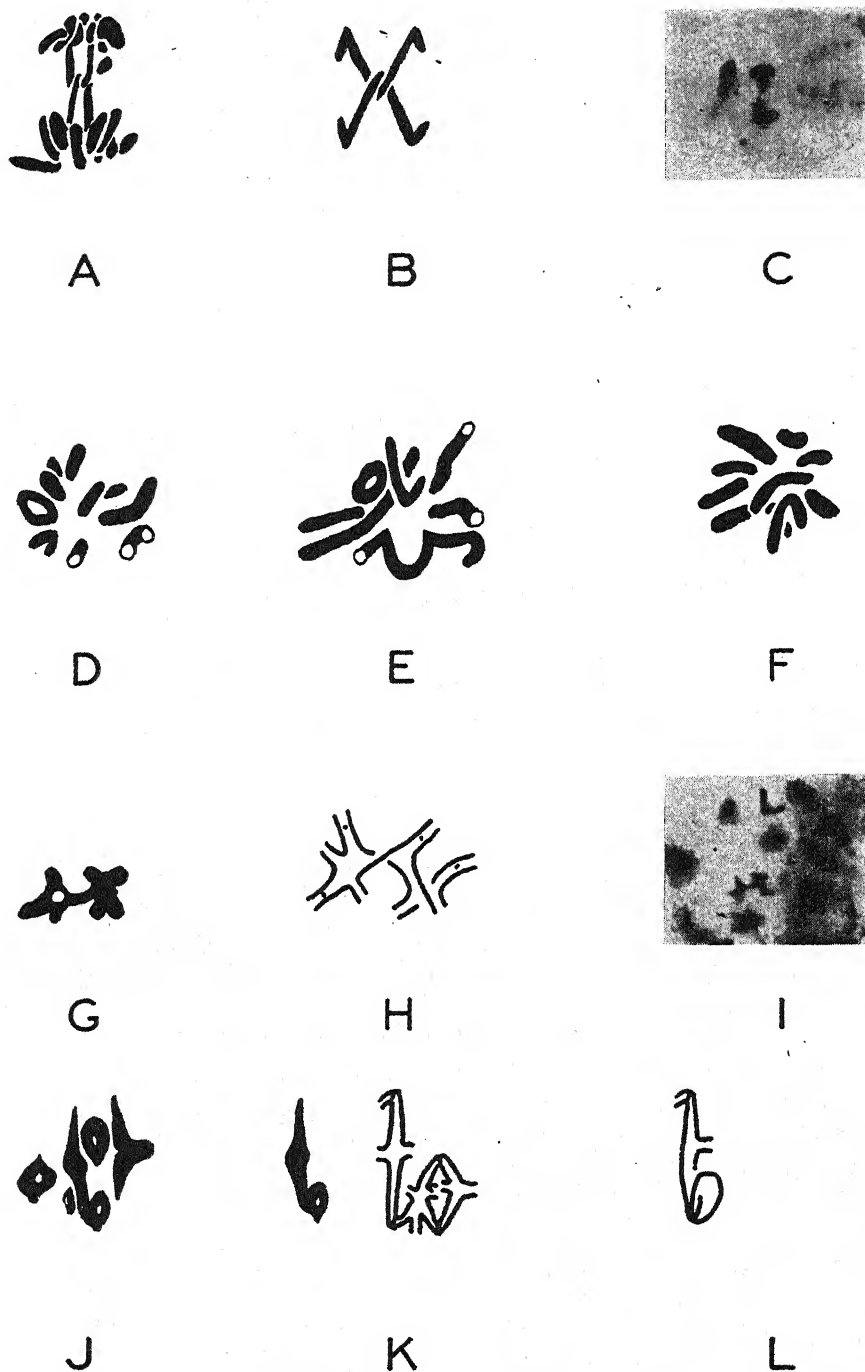


FIG. 10. Description: Aberrant configurations in a strobila of *Hymenolepis anthocephalus* are shown, from top to bottom, in anaphase of mitosis A, metaphase of mitosis D, E, F, diplotene of meiosis G, I, and metaphase of meiosis J. Diagrams illustrate interlocking dicentric chromosomes B, a quadrivalent association at diplotene H, and two possible explanations of the ring-rod configuration at first metaphase K and L. The photographs portray the actual configurations drawn. Magnification of drawings ca. 4800 $\times$ . Magnification of photographs ca. 2300 $\times$ .

nucleus, broken ends of one may unite with those of another, forming a dicentric chromosome. When a dicentric chromosome of whatever origin divides at mitosis, it, like a ring, may separate into free and equal parts; or it may interlock in the region between the centromeres, and form a bridge at anaphase. The bridge shown in Fig. 10, A, B, C, is probably of the latter type. Breakage of a dicentric into two chromosomes, by the breaking of such a bridge, will almost certainly leave in one of the parts a segment translocated from the other part. Thus the quadrivalent association shown in Fig. 10, G, may be thought of as the result of one or more such translocations. The ring-rod configuration (Fig. 10, J) seen at first metaphase may be, however, merely the product of simple pairing of a persistent ring with its rod homologue. A single chiasma at pachytene could produce the configuration seen at metaphase (Fig. 10, L).

It is apparent from the foregoing account that the ring chromosomes seen at mitotic metaphase in *Hymenolepis anthocephalus* are probably of the same sort as those seen in maize. The explanation of aberrant chromosomal configurations in maize serves to explain the same kinds of aberrations in the cestode.

2. *Hymenolepis diminuta* (Rud.) Blanchard, 1891, from rats and mice, was very difficult to fix well. It is not represented in the plates because no very good mitoses or meioses could be found. The number of chromosomes is probably 12, but a few rather poor aceto-carmine smears furnished the only mitotic data. The worm can be fixed well, perhaps in the Bhaduri and Semmens fixative, which has not yet been thoroughly tested on cestode material.

3. *Aploparaksis* Clerc. 1903, is a genus represented in this study by a fragment of one cestode, recovered from the intestine of a starling along with several specimens of *Hymenolepis serpentulus* (Schrank) Cohn, 1901. Upon reconstruction of serial sections, this worm (which looked very much like *H. serpentulus*) was found to have but one testis *per* proglottid. Hence it must be placed in the above genus. Further identification could not well be made, as the scolex was missing.

The chromosomes of this cestode look rather like those of *H. serpentulus sturni* (the temporary subspecific is the writer's designation used to distinguish this worm from one of the same species found in the robin, to be discussed later), the cestode with which it was found (Figs. 6, 7). The latter lacks the pair of large chromosomes with median constrictions, as well as the heteromorphic chromosomes found in the *Aploparaksis* species; but the "general morphological similarity" between the chromosomes of both worms, in mitosis and meiosis, is pronounced. The heteromorphic chromosome is not as constant as that found in *Protogynella blarinae* (see above) but appears often enough to be considered characteristic.

4. *Hymenolepis serpentulus* (Schrank) Cohn, 1901, is represented here by several strobilae recovered from the common starling, as well as by several from the robin. Since there are cytological differences between the two forms, as well as slight morphological differences, the worms will be taken up separately. Awaiting a study of the type for the species, and the collection of more specimens from each host, the author proposes, solely for present purposes, to designate the two cestodes by subspecific names.

*Hymenolepis serpentulus sturni*, the starling's parasite, has four identical pairs (or eight morphologically similar chromosomes) with median centromeres, and two slightly different pairs of smaller chromosomes (Fig. 7). This complement is



unique among the cytologically known HYMENOLEPIDIDAE, in that median constrictions are not elsewhere so frequent.

The chiasma frequency of *H. serpentulus sturni* can be given only as a minimum frequency, based on univalent occurrence. In 6 out of 106 cells studied at meiotic metaphase I, the chromosomes are present as four bivalents plus one quadrivalent. The quadrivalent (if such it is) is always a chain of four chromosomes, except in one case where a ring was found. Considering the doubtful configuration as a quadrivalent,<sup>4</sup> the minimum chiasma frequency becomes 1.01 per bivalent, or 6.06 per cell. It is probable that this figure is near the actual, but the prophase fixation was not good enough to permit analysis of bivalents.

5. *Hymenolepis serpentulus turdi*, the cestode recovered from the robin, is morphologically similar to the cestodes of the same species recovered from the starling. There are, however, differences in dimensions which may have taxonomic significance, if found, from extensive collections, to exist as constant distinguishable features. The rostellar hooks, 10 in number in each cestode, are about 8–10% shorter in the robin cestode; their shape is the same, however, in both subspecies. The scolex of the robin cestode is likewise about 8–10% smaller in its linear dimensions. The neck is more slender. The dimensions of the proglottids are about the same for corresponding regions of the strobila in both. Internal anatomy is not different. The strobila of *H. serpentulus sturni* is probably over all longer than that of *H. s. turdi*, but the given range of variation in Cohn's description probably permits such differences. On the basis of material at hand (eight strobilae from the starling, and four from the robin) it does not seem wise to break down the species, or to describe one of the forms as new. The fact that at least twenty-five separate references to this species occur in the literature<sup>5</sup> (as compiled by Hughes, 1940a) makes the problem of variation within the species an interesting one.

Cytologically, the two cestodes show definite differences. *H. serpentulus sturni* (Fig. 7) has four almost identical pairs of small, medially constricted chromosomes, these being slightly larger than the two other pairs. *H. s. turdi* (Fig. 8) has one large pair with subterminal centromeres, three smaller pairs (still larger than any of the chromosomes of *H. s. sturni*) with subterminal constrictions, a single smaller pair of the same type, and a single pair of chromosomes about the size of the small chromosomes of *H. s. sturni*. This cytological difference is probably significant. Although the size of chromosomes is a variable thing, even within an individual, the morphology of chromosomes, as evidenced by centromere location, is not thought to be variable in genetically homogeneous individuals or populations. When structural diversity is observed between the chromosome complements of two organisms, genetic diversity may be presumed. In the narrowest sense this must be true, whatever the phenotypic evidence, since it is well known that, in many organisms, the mere inversion or translocation of parts of a chromosome is a permanent

<sup>4</sup> It is the writer's opinion that what he sees in this material is an association of more than two chromosomes. But the possibility of fusion artifact must be considered before any conclusion is drawn. Those interested may look again at Fig. 7, and note the appearance of chromosome reduplication, as if a base number of three had become involved in tetraploidy. As White (1940) pointed out in his survey report of the chromosomes of hermaphrodites, there seems to be no reason why hermaphroditic animals should not show the same range of polyploidy found in the higher plants.

change in the organism which may be demonstrated cytologically. It is almost axiomatic that genetic mutation has its seat in the chromosome; thus where chromosomes of otherwise similar organisms differ by structural mutation, one can say that in that respect at least, and also, probably, in physiological respects as well, the two organisms are different. On a cytological basis, therefore, the two types of *H. serpentulus* can be distinguished. But certainly the taxonomic value of cytological data is still very slight in most fields of investigation, in the practical uses to which taxonomy must be put. It might be unwise to attempt formally to split the recognized species into two subspecies or varieties, on the basis of the slight morphological evidence presented in the preceding paragraph, and upon the evidence just presented concerning cytological differences.

*Conclusion: the Hymenolepididae*

From the detailed description of the chromosomes of nine species from the family HYMENOLEPIDIDAE, a few general conclusions may be drawn. First, so far as the present study goes, cestodes of this family show considerable uniformity in number and type of chromosomes. Fig. 11 is an illustration of how the chromosome numbers and morphologies in the HYMENOLEPIDIDAE are distributed more or less continuously among the taxonomic groups. For added interest, the scoleces, at various magnifications, are sketched beside the chromosomes of each species. This illustration suggests that the HYMENOLEPIDIDAE are a homogeneous group, in which generic divisions are perhaps formal. While it cannot be said, from present data, how far this uniformity extends throughout the whole family, it can be stated with conviction that, cytologically, the representatives studied are similar.

IV. CHROMOSOMES OF THE FAMILY DILEPIDIDAE

Six species from the family DILEPIDIDAE were found suitable for chromosome studies. Three of these belong to the subfamily PARAUTERININAE Fuhrmann and three to the subfamily DIPYLIDIINAE Stiles. (Several other cestodes of this family and of the families ANOPOLOCEPHALIDAE Fuhrmann and AMABILIDAE Braun were obtained; but no accurate chromosome number or determinations on morphology could be made, either because the cestodes were dead when recovered, or because the fixation technique failed.)

1. *Rhabdometra similis* Ransom, 1909, from the hairy woodpecker (a new host record for this cestode), has a diploid complement of 12. There are (see Fig. 12) a large pair with subterminal centromeres, another pair of the same size with median centromeres, a smaller pair with median centromeres and three pairs of still smaller chromosomes.

The chromosomes of *R. similis* are the smallest yet found in the DILEPIDIDAE. Probably those of *Anonchotaenia globata* (?) (Fig. 13) are actually as small; but the figures for the latter were taken from aceto-carmine preparations, in which the chromosomes are generally one-third larger than they appear in other preparations. The structural differences between the chromosomes of *R. similis* and those of *A. globata* are not marked.

2. *Anonchotaenia globata* (von Linstow), Joyeux and Baer, 1936, from the phoebe, is both cytologically and morphologically close to *R. similis*. Its chromosomes (Fig. 13) consist of one large pair with median centromeres, one smaller

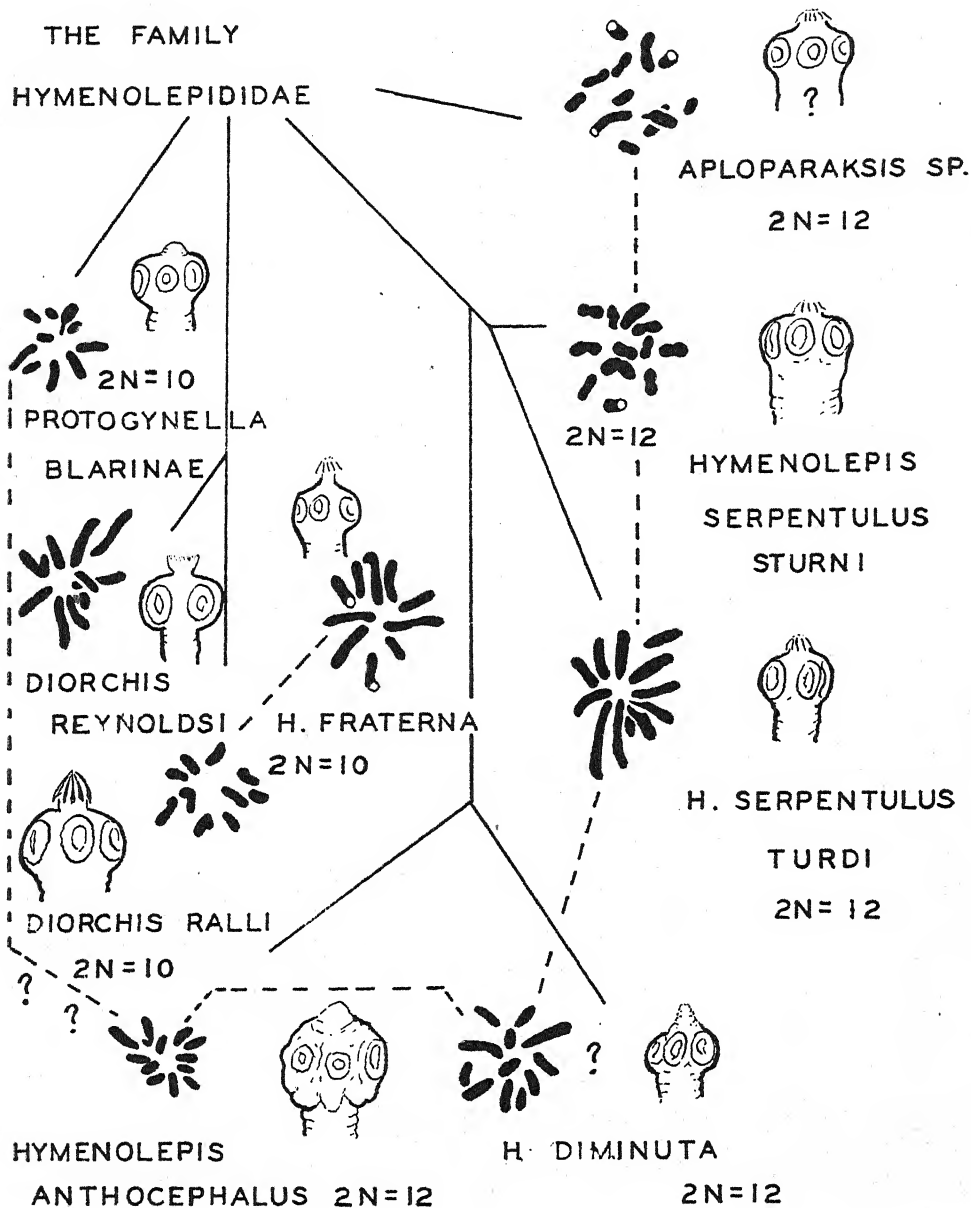


FIG. 11. Description: Four genera of the family are represented by nine complements. The scolex of each worm is sketched beside its chromosomes. Straight lines indicate probable affinities, on morphological grounds. Broken lines indicate probable affinities, on cytological grounds. The line between *D. reynoldsi* and *H. anthocephalus* is highly problematical, suggested by the univalent occurrence in *D. reynoldsi* as a possible means of change in chromosome number. In general, chromosomal and morphological characters overreach generic limits, and support the suggestion that this family is relatively uniform. Magnification of chromosomes ca. 3600 $\times$ . Magnification of scoleces, 22.5 $\times$  to 45 $\times$ .

## MITOSIS AND MEIOSIS

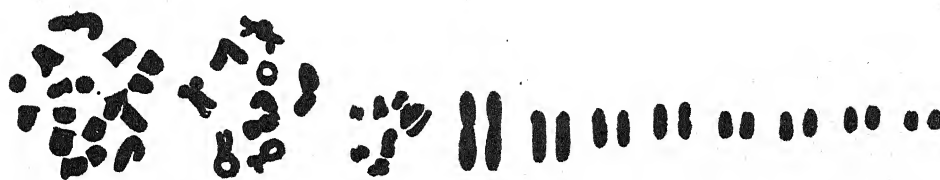
## IDIOGRAM



12



13



14



15

FIGS. 12-15. Description: All figures ca. 4000  $\times$ .

FIG. 12. *Rhabdometra similis*: Mitosis, meiosis (I and II) and idiogram.

FIG. 13. *Anonchotaenia globata*: Mitosis and idiogram. This drawing was taken from an aceto-carmin preparation, which probably caused considerable swelling. Fixation in LaCour's 2 BE, which was used for the preparations drawn in Fig. 12, would have resulted in chromosomes of comparable size.

FIG. 14. *Anonchotaenia* sp.: Mitosis (early anaphase), meiosis I (diakinesis), meiosis II, and idiogram. Note the similarity in type to chromosomes of Figs. 12 and 13. The reduplication of four small chromosomes in 12 or 13 would result in a complement like 14.

FIG. 15. *Monophylidium* sp.: Mitosis, meiosis (I and II) and idiogram. Note dissimilarity of this to three preceding, and similarity to Fig. 16.

pair with median centromeres, a middle-sized pair, with subterminal centromeres and three smaller pairs. A comparison of the idiograms in Figs. 12 and 13 shows how alike the two complements are.

The scolex of *A. globata*, illustrated in Fig. 20, is probably similar in form to the normal scolex of *R. similis*. The type for the latter lacked a scolex. A scolex was present in the complete cestode recovered by the author, but it possessed only three suckers, instead of the four found throughout the whole order CYCLOPHYLIDEA, to which these cestodes certainly belong. Sectioned and stained for histological details, this scolex showed that the fourth sucker was present only as a sclerotic mass of cells lying beneath the cuticula, surrounded by rudimentary protractor and retractor muscles. It is probable that this scolex was "abnormal"; yet its functional parts suggest the hypothetical normal scolex of *R. similis* (as drawn in Fig. 20). A note has been prepared, emending Ransom's description of the species.

3. Four strobilae of another species of *Anonchotaenia* were recovered from a golden-crowned kinglet. This cestode, not yet positively identified as to species, is clearly distinct from *A. globata* because of the unusual vermiform, hookless (?) embryos contained in its ellipsoidal eggs.

Its chromosomes are shown in Fig. 14. They are seen to be 16 in number, with the large medially constricted pair and the other large pair noticeable in the idiograms of the two preceding species (Figs. 12 and 13). In addition to these two pairs, there are six pairs of smaller, not uniform, but hardly distinguishable, chromosomes.

4. A specimen of *Liga brasiliensis* Ransom, 1909, formerly reported (Ransom, 1909) from the golden-winged woodpecker, was recovered from the hairy woodpecker. Its diploid complement of 14 chromosomes was studied mainly in cleavage divisions of the eggs. The morphology of the complement can be seen (Fig. 16) to be similar to that of *Monopylidium* sp. (Fig. 15). Add one pair of the smallest type chromosome to the idiogram of *Liga*, and it will be like that of *Monopylidium*, except for size and the relative lengths of the large subterminals in each species.

5. A single complete worm, identified as to genus as *Choanotaenia* Railliet, 1896 (= *Monopylidium* Fuhrmann, 1899), recovered from the robin, has perhaps the largest chromosomes yet found in the cyclophyllidean cestodes. This worm has a diploid complement of 16. There are (Fig. 15) a large pair with submedian centromeres, a large pair with subterminal centromeres, a slightly smaller pair with submedian centromeres, a still smaller pair with submedian centromeres, and four small pairs with submedian centromeres.

Morphologically, this worm is close to the genus *Liga* (see sketches of respective scoleces in Fig. 20). Its general shape and size are different, and the genital systems are unilateral, not alternating as in *Liga*; but the smaller anatomical details, such as testis number and structure, ova nutrition in parenchymatous capsules, etc., show the morphological relationships to be close. Its chromosome number, sixteen, to *Liga*'s fourteen, is a difference rather insignificant when weighed against the marked similarities between the complements.

6. *Dipylidium caninum* (Linné, 1758),<sup>5</sup> the common dog and cat tapeworm,

<sup>5</sup> This cestode species has been the subject of considerable controversy, some workers, e.g., Millzner (1926), stating that the dog-cat tapeworms of this genus constitute many species, others, e.g., Venard (1938), reducing the number of species to one or a few. Since the controversy will resolve itself into merely difference of opinion, unless some new taxonomic criteria



is worth a special study, which the author has not had time to make. The chromosomes (see Fig. 17) probably number 10 diploid. The idiogram is in this case derived solely from meiotic stages, and may, therefore, be inaccurate.

#### Conclusions: the Dilepididae

The descriptive data given above suggest two tentative conclusions. First, the wide range of chromosome numbers (four) in the DILEPIDIDAE, when compared with the narrow range (two) found in the HYMENOLEPIDIDAE, indicates that the DILEPIDIDAE are cytologically diverse. The conclusion is admittedly based on data whose sampling error may be very large; each group studied represents a very small percentage (ca. 2.0%) of the known numbers of species in the family. But a study of even such small portions of rather well defined groups of organisms will be more likely to reveal a trend which exists in the whole of the groups, than to simulate a trend which does not exist beyond the sample. Second, the chromosomes of the DILEPIDIDAE, as here reported, support rather than question the taxonomic ordering of that family, as regards the two subfamilies DIPYLIDIINAE and PARAUTERININAE. In the first subfamily, the cestodes *Monopylidium* sp. and *Liga brasiliensis* show both morphological and chromosomal affinity. The other member of that subfamily, *Dipylidium caninum*, although its chromosome number is only 10, as against the others' 16 and 14, is probably not out of place, cytotaxonomically, in their company. In the second subfamily, the PARAUTERININAE, the two cestodes *Rhabdometra similis* and *Anonchotaenia globata* are almost identical, cytologically. Their morphological characters are also similar, the main generic difference being that in *R. similis* the genital ducts pass between the excretory canals, while in *Anonchotaenia* they pass dorsal to the canals. The other species of *Anonchotaenia*, while it has four more chromosomes than the other two, and differs from them radically in the embryo stage, shows chromosome structure that follows the idiogrammatic pattern set by them. Figure 20 illustrates the subfamily coherence evinced in both cytological and morphological ways by the cytologically known DILEPIDIDAE.

#### V. GENERAL CONCLUSIONS AND SUMMARY

The above study suggests that the HYMENOLEPIDIDAE are cytologically uniform, the DILEPIDIDAE cytologically diverse. These qualities are not restricted to the chromosomal aspects of the two groups, but extend to other aspects. First, data obtained from the taxonomic arrangement within the two groups indicates that the HYMENOLEPIDIDAE have few genera but many species, while the DILEPIDIDAE have many genera and few species (see Fig. 21). Since the same taxonomists have studied both groups, and to approximately the same extent, the above taxonomic fact can safely be considered a morphological one; i.e., where generic differences

---

can be put to use, a cytological comparison of the disputed species is desirable. Venard (1938) approached the problem intelligently when he undertook to control genetically and physiologically the rearing of many strobilae of *Dipylidium caninum*, in laboratory animals, from the eggs of a single proglottid. As Stunkard suggested to the author, however, Venard failed to eliminate the possibility of obtaining a heterozygous progeny as the result of univalent occurrence, hybridity, or other cytological means of large-scale segregation. The fact that Venard obtained, in the experimental progeny, most of Millzner's species, does not, therefore, conclusively show that the difference between these "species" is due merely to environmental influence. A cytological study of that progeny might have resulted in different conclusions.

## MITOSIS AND MEIOSIS

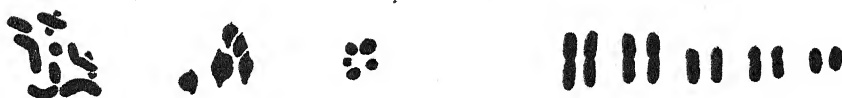
## IDIOGRAM



16



17



18



19

FIGS. 16-19. Description: Figures 16-18 ca. 4030 $\times$ ; Fig. 19, ca. 2015 $\times$ .

FIG. 16. *Liga brasiliensis*: Mitosis (early anaphase), meiosis (I and II), and idiogram. Six bivalents are shown in meiosis (metaphase I), with the seventh bivalent probably already separated. Note similarity in chromosome type to Fig. 15.

FIG. 17. *Dipylidium caninum*: Meiosis (diakinesis), idiogram. The information must be considered tentative only, since little material was examined. The idiogram is derived from meiotic configurations and is not reliable.

FIG. 18. *Oochoristica* sp. (Fam. Anoplocephalidae): Mitosis, meiosis (I and II), and idiogram. An incidental report of chromosome number  $2n=10$ .

FIG. 19. *Archigetes appendiculatus* Ratzel: Mitosis, meiosis (I and II), with idiogram. Adapted from Motomura's original drawings. Idiogram derived from Motomura's figures of mitotic metaphases.

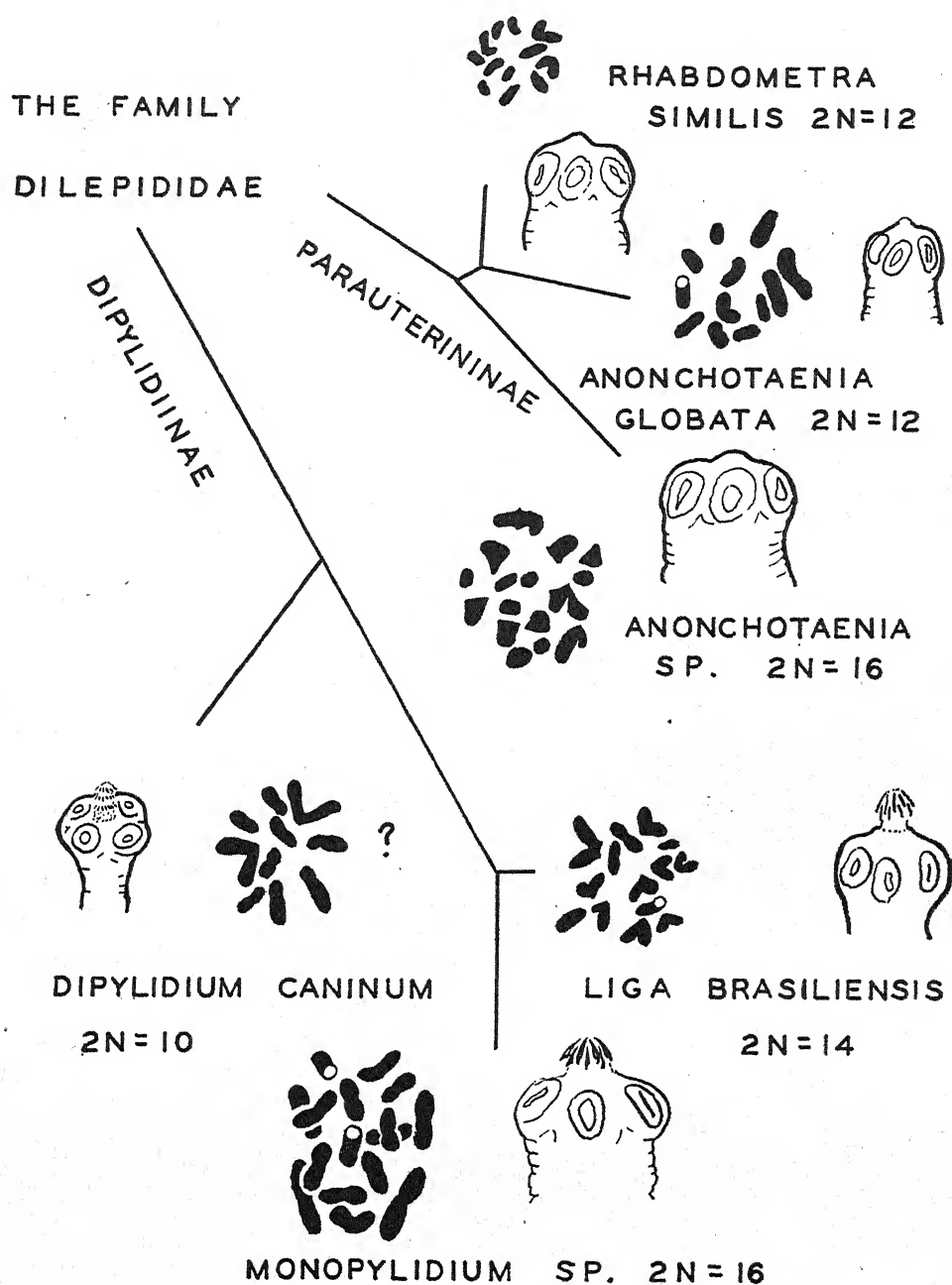


FIG. 20. Description: Five genera of the family are represented by six chromosome complements. The scoleces are sketched beside the chromosomes. Chromosome morphology and number support the taxonomic division into two subfamilies. The type of scolex, especially in respect to the rostellum (armed, in the DIPYLIDIINAE, with two or more rows of hooks), supports the same subdivision. In general, chromosomal and morphological diversity in the family is illustrated. Magnification of chromosomes ca. 3800 $\times$ . Magnification of scoleces, 23.7 $\times$  to 47.5 $\times$ .

are few, as in the HYMENOLEPIDIDAE, the morphology is uniform, while where generic differences are many, as in the DILEPIDIDAE, the morphology is diverse.

Second, ecological data confirm the above cytotaxonomic coincidence. The greater part of the HYMENOLEPIDIDAE (see Hughes, 1940a) are parasites of the avian orders ANSERIFORMES, CHARADRIIFORMES, and COLYMBIFORMES—shore or water birds; while the DILEPIDIDAE (see Joyeux and Baer, 1936) are parasites of the order PASSERIFORMES—land birds of the most varied habits of feeding and nesting. The hosts of the HYMENOLEPIDIDAE, a morphologically uniform group of parasites, are ecologically uniform; while the hosts of the DILEPIDIDAE, morphologically diverse parasites, are ecologically diverse. On ecological grounds, therefore, it may again be said that the HYMENOLEPIDIDAE are uniform, the DILEPIDIDAE, diverse.

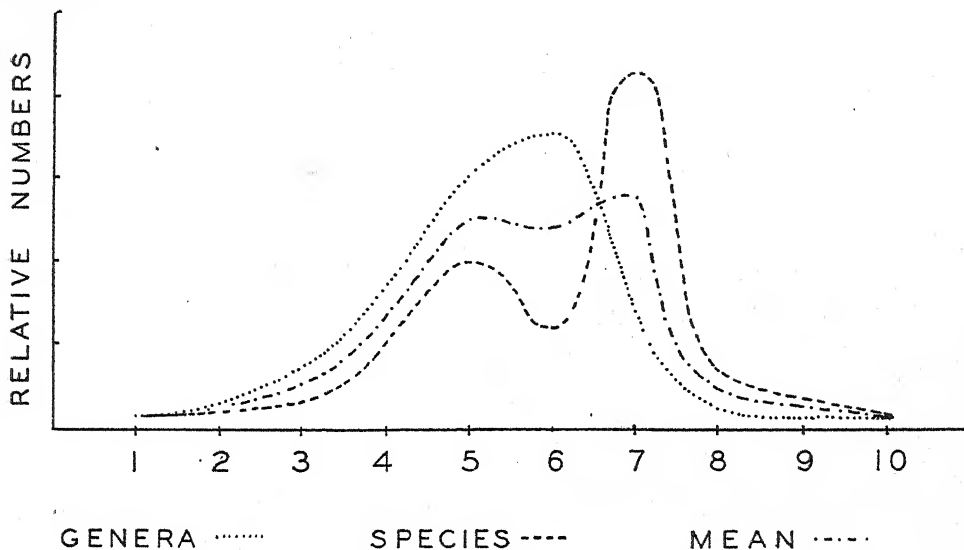


FIG. 21. Illustrative graph showing the families of cyclophyllidean cestodes grouped according to numbers of species (dash line) and genera (dot line) with an arbitrary mean or average (dash and dot line). The relative uniformities of the families can be read as functions of the differences between species and genera frequencies. Family 1, MESOCESTOIDIDAE; 2, AMABILIDAE; 3, ACOLEIDAE; 4, DAVAINIDAE; 5, DILEPIDIDAE; 6, ANOPLOCEPHALIDAE; 7, HYMENOLEPIDIDAE; 8, TETRABOTHRIDAE; 9, TAENIIDAE; 10, NEMATOTAENIIDAE. (See Hughes, 1940a, 1940b, 1942; Joyeux and Baer, 1936.)

The cytological results of the present partial survey have indicated the same uniformity and diversity. In number and morphology the chromosomes of the hymenolepidid worms are very similar. The idiograms look alike; the numbers found are 10 and 12 diploid, only; and the "general appearance" of the chromosomes of these species shows uniformity. (Chiasma frequency data, since none was obtained from the other family, need not be considered in this respect.) In the DILEPIDIDAE, on the other hand, the chromosomes are varied in number and morphology. Idiograms do not look alike; the numbers found are 10, 12, 14 and 16 diploid; and the "general appearance" of the chromosomes shows diversity. It may be stated, therefore, on the basis of the data at hand, that the chromosome complements of the HYMENOLEPIDIDAE are relatively uniform, while the complements of the DILEPIDIDAE are relatively diverse.

A single hypothesis arises from consideration of the above taxonomic, ecological and cytological conclusions. It is the idea that, in two taxonomic divisions of the cyclophyllidean cestodes, there is coincidence of cytological, ecological, and morphological descriptive data; it is the suggestion that a system of cestode classification may be, by the demonstration of such coincidence, supported as a "natural" system.

## SUMMARY

1. The literature on cestode cytology reveals inconclusive results, which in general suggest that cestode nuclei behave differently from nuclei of other organisms. No recent work had been done in this field before the present study.

2. Techniques of collection, fixation, and staining are described. Cestodes, obtained alive from birds, mammals, and amphibians, were found to be best fixed cytologically by acetic-alcohol solutions, osmic-chromic solutions, or sodium diuranate solutions. Sectioning a 14  $\mu$  is recommended. Smear or squash technique, with the Feulgen stain, is recommended also. Preferred stains for sections are Newton's crystal-violet-iodine or the Feulgen reagent.

3. The chromosome numbers and morphologies of fifteen cestodes are given. Numbers in the HYMENOLEPIDIDAE are ten and twelve, those in the DILEPIDIDAE are ten, twelve, fourteen and sixteen. An aberration in *Hymenolepis anthocephalus* is described and related to the ring chromosome aberrations in maize. Occurrence of univalents is noted and compared in several cestodes. Chiasma frequencies are reported where possible.

4. Cytologically, the observed portion of the HYMENOLEPIDIDAE is shown to be uniform, while the observed portion of the DILEPIDIDAE is diverse. The families HYMENOLEPIDIDAE and DILEPIDIDAE are discussed in a taxonomic light, on the basis of species- and genera-frequencies, and in an ecological light, on the basis of host uniformity, and are shown to have the same uniformity and diversity established cytologically.

## REFERENCES

- BHADURI, P. N. AND SEMMENS, C. S. 1942 II. Nucleolar staining method applied to animal tissues. J. Roy. Micr. Soc., series 3, 60: 21-24.
- CHILD, C. M. 1904 On amitosis in *Moniezia*. Anat. Anz. 25: 545-548.
- 1911 The occurrence of amitosis in *Moniezia*. Biol. Bull. 21: 280-296.
- DARLINGTON, C. D. 1937 Recent advances in cytology. 2nd ed. The Blakiston Co. Phila.
- HARMAN, M. T. 1913 Method of cell division in the sex cells of *Taenia teniaeformis*. J. Morph. 24: 205-242.
- HEITZ, E. 1935 Die Nukleal-Quetschmethode. Deutsch. Bot. Gesell. 53: 870-878.
- HUGHES, R. C. 1940a The genus *Hymenolepis* Weinland, 1858. Tech. Bull. No. 8, Okla. Agric. and Mech. College Exp. Stat. 42 pp.
- 1940b The genus *Oochoristica* Lühe, 1898. Am. Midl. Nat. 23: 368-381.
- 1942 The genus *Railletina* Fuhrmann, 1910. Bull. Oklahoma Agric. and Mech. College 29: 1-53.
- HUSTED, L. AND BURCH, P. R. 1943 Chromosomes of Virginia snails. I. Polygyridae (Abst.) Proc. Va. Acad. Sci. 1943-44: 44.
- JONES, A. W. 1943a *Protogynella blarinae* n.g. n.sp.; a new cestode from the shrew *Blarina brevicauda* Say. Tr. Am. Micr. Soc. 2: 169-173.
- 1943b *Pseudostomum caccum* (von Graff, 1883) n. comb.; morphological and cytological studies on an alloecocoele-turbellarian. J. Morph. 73: 313-327.
- JOYEUX, C. AND BAER, J. G. 1936 Cestodes. Faune de France 30: 1-613.
- KOLLER, P. C. AND DARLINGTON, C. D. 1934 The genetical and mechanical properties of the sex chromosomes. I. *Rattus norvegicus*, ♂. J. Genet. 29: 159-173.
- LACOUR, L. 1931 Improvements in everyday technique in plant cytology. J. Roy. Micr. Soc. 51: 119-126.



- 1937 Plant cytological technique. Bot. Rev. 5: 241-258.
- LARSH, J. E., JR. 1943 The relationship between intestinal size of young mice and their susceptibility to infection with the cestode *Hymenolepis nana* var. *fraterna*. J. Parasitol. 29: 61-64.
- 1944 Comparative studies on a mouse strain of *Hymenolepis nana* var. *fraterna*, in different species and varieties of mice. J. Parasitol. 30: 21-25.
- LÜHE, M. 1910 Parasitische Plattwürmer II. Cestodes. In Brauer's Süßwasserfauna Deutsch. 18: 153 pp.
- McCLINTOCK, B. 1932 A correlation of ring-shaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. 18: 677-681.
- 1938a The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics 23: 312-376.
- 1938b The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. Univ. of Missouri Coll. of Agric. Agric. Res. Stat. Res. Bull. 290.
- 1939 The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc. Nat. Acad. Sci. 25: 405-415.
- MILLZNER, T. M. 1926 On the cestode genus *Dipylidium* from cats and dogs. Univ. Calif. Publ. Zool. 28: 317-356.
- MOTOMURA, I. 1929 On the early development of monozoic cestode, *Archigetes appendiculatus*, including the oogenesis and fertilization. Annot. Zool. Japon. 12: 109-129.
- NEWTON, W. C. F. AND DARLINGTON, C. D. 1929 Meiosis in polyploids I. J. Genet. 21: 1-16.
- PAINTER, T. S. 1939 An acetocarmine method for bird and mammalian chromosomes. Science 90: 307-308.
- PENNYPACKER, M. I. 1940 The chromosomes and extranuclear material in the maturing germ cells of a frog lung-fluke, *Pneumonoeces similiplexus* Stafford. J. Morph. 66: 481-495.
- RANSOM, B. H. 1909 The taenoid cestodes of North American birds. U. S. Nat. Mus. Bull. 69: 141 pp.
- REES, G. 1939 Studies on the germ cell cycle of the digenetic trematode *Parorchis acanthus* Nicoll. I. Anatomy of the genitalia and gametogenesis in the adult. Parasitology 31: 417-433.
- RICHARDS, A. 1911 The method of cell division in the development of the female sex organs of *Moniezia*. Biol. Bull. 20: 123-178.
- SNYDER, L. H. 1940 The principles of heredity. D. C. Heath & Co. New York.
- VENARD, C. E. 1938 Morphology, bionomics, and taxonomy of the cestode *Dipylidium caninum*. Ann. N. Y. Acad. Sci. 37: 273-328.
- WHITE, M. J. D. 1940 Evidence for polyploidy in the hermaphrodite groups of animals. Nature 146: 132-133.
- YOUNG, R. T. 1910 The somatic nuclei of certain cestodes. Arch. Zellforsch. 6: 140-163.
- 1912 Cytology of cestoda. Verhandl. 8 Internat. Zool-Kong. zu Graz.
- 1923 Gametogenesis in cestodes. Arch. Zellforsch. 16: 419-437.

## A NEW ANOPHELINE FROM THE SOLOMON ISLANDS WITH NOTES ON ITS BIOLOGY

CAPT. WILLIAM B. OWEN, Sanitary Corps, AUS

A study of the biologies of the anopheline mosquitoes of the Koli District, Guadalcanal Island, British Solomon Islands Protectorate, revealed the presence of a species that is considered new and is here described under the name *Anopheles koliensis*. A summary of the information on its biology is presented.

Acknowledgment is made to Captain Louis J. Olivier, Sanitary Corps, AUS, for assistance in the preparation of the illustrations, Sgt. Kenneth H. Miller, Medical Department, AUS, assisted with the laboratory studies.

### *Anopheles koliensis* n. sp.

**ADULT FEMALE.**—A medium-sized, yellowish anopheline with a patch of white scales on the ventral surface of the apical third of the labium.

**Head:** Frontal tuft prominent, white; vertical setae white with admixture of white hair-like scales; top of vertex with white erect scales forming lateral spots, remainder of vertex and occiput covered with erect dark scales. Ocular setae 6. Antenna with minute white scales on third segment. Palpi ornamented as in plate I, markings slightly variable, light scales yellowish to white. Labium black except for patch of white scales on ventral surface of apical third. size of this patch slightly variable, in some specimens visible from above at distal end.

**Thorax:** Anterior promontory with sparse light scales in center; lateral dense, light with a few dark scales below. Remainder of mesonotum with vestiture of yellowish white scales. Mesonotal bristles golden brown, integument gray with dark eye spot anterior to scutal angle. Pleura dark with evidence of a wide black line dorsally. Spiracular bristle absent, propleurals 4-6, lower sternopleurals 4, upper sternopleurals 6, prealars 8, subalars 7-9, lower mesepimerals absent.

**Wing** (Plate I): As shown in diagram. Scales rather narrow; pale areas light yellow. Dark spots on costal margin constant; median and subapical dark spots may extend to vein 1. Markings on other veins somewhat variable; distal half of 4th vein darker than other veins; fringe spots constant except dark spot at apex which is variable in size.

**Legs** (Plate I): Front femora swollen on basal half, uniformly speckled; middle and hind femora speckled, lighter on inner surfaces. All tibia speckled, lighter on inner surfaces. Front tarsus with first segment light at apex and variable number of light spots; second and third segments with basal and apical light bands; fourth with basal light band; fifth dark. First segments of middle and hind tarsi similar to corresponding segment of front tarsus, second, third and fourth segments with small apical light bands, fifth all dark. Second segment of middle and hind tarsi with one or two light spots in middle.

**Abdomen:** Abdominal vestiture of golden hairs with scales limited to seventh and eighth segments. Seventh segment with few yellow scales. Eighth segment clothed with yellow scales and lateral patch of dark scales at apex. Cerci with yellow scales at base, dark at apex.

**ADULT MALE.**—General coloration of the female. Proboscis all black. Fourth segment of antenna with small tuft of narrow white scales. First segment of palpus with few white scales in middle, second with median and apical white bands, third and fourth with apical halves white.

**LARVA.—Head** (Plate II): Inner clypeals widely separated, slender, without branches. Outer clypeals unbranched, about two-thirds length of inner. Posterior clypeals small, unbranched. Frontal hairs with branches as shown in plate. Occipitals short, three to four branches. Orbitals minute unbranched. Antenna with minute spines, antennal hair small, unbranched, arising one-third distance from base; terminal hair with three to five branches arising from base.

**Thorax** (Plate II): Prothoracic hair 1 with small basal tubercle, swollen shaft, six to eight radiating branches on each side, shorter than hair 2. Basal tubercles of 1 and 2 separate. Hair 2 approximately two-thirds length of hair 4, with small tubercle, slender shaft, six to seven branches on each side. Hair 3 simple. Hair 4 with six to eight prominent lateral branches. Hair 5 longer than 4, shaft more slender with short lateral branches. Hair 6 simple, slender, longer than 5. Hair 7 with slender shaft, longer than 6, many long radiating

branches. Prothoracic pleural hair 8 long with many branches; 9–12 simple. Mesothoracic hair 1 with thick shaft and eight to ten radiating branches on each side. Metathoracic hair 1 forming a rudimentary palmate hair with five to eight leaflets.

*Abdomen* (Plate II): Palmate hair on I poorly developed; II to VII well developed (14–16 leaflets); weaker on II. Leaflets (Plate II) pigmented; filaments tapering with two or three indentations on each side. Lateral hairs on III trifurcate, IV and V bifurcate. Anterior tergal plates well developed. Pecten (Plate II) with usually thirteen spines, three or four of these long, remainder medium in length; fine serrations at base of spines. Pecten hair with four or five branches.

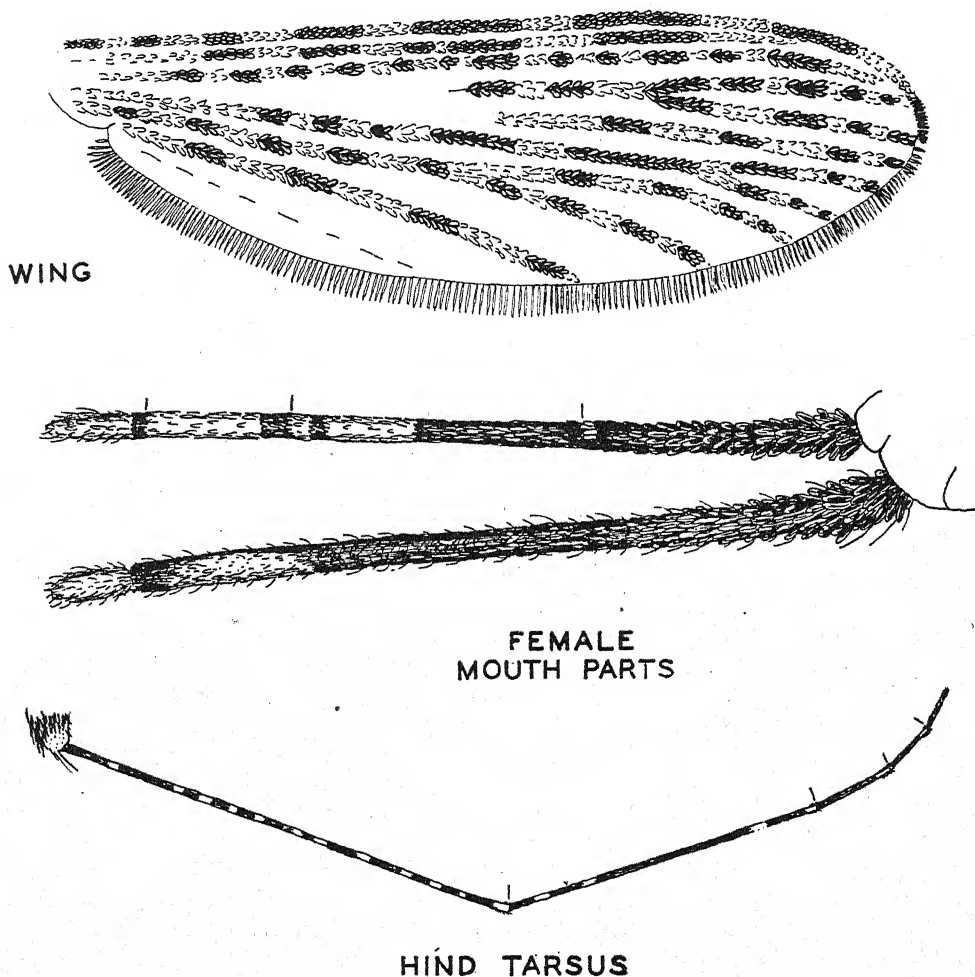


PLATE I  
Morphology of adult *Anopheles koliensis*

**TYPES.**—Holotype female, allotype male reared in laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series A: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 6 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series B: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 4 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 4, 1944 (Owen). Paratype series C: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 8 females, 3 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype

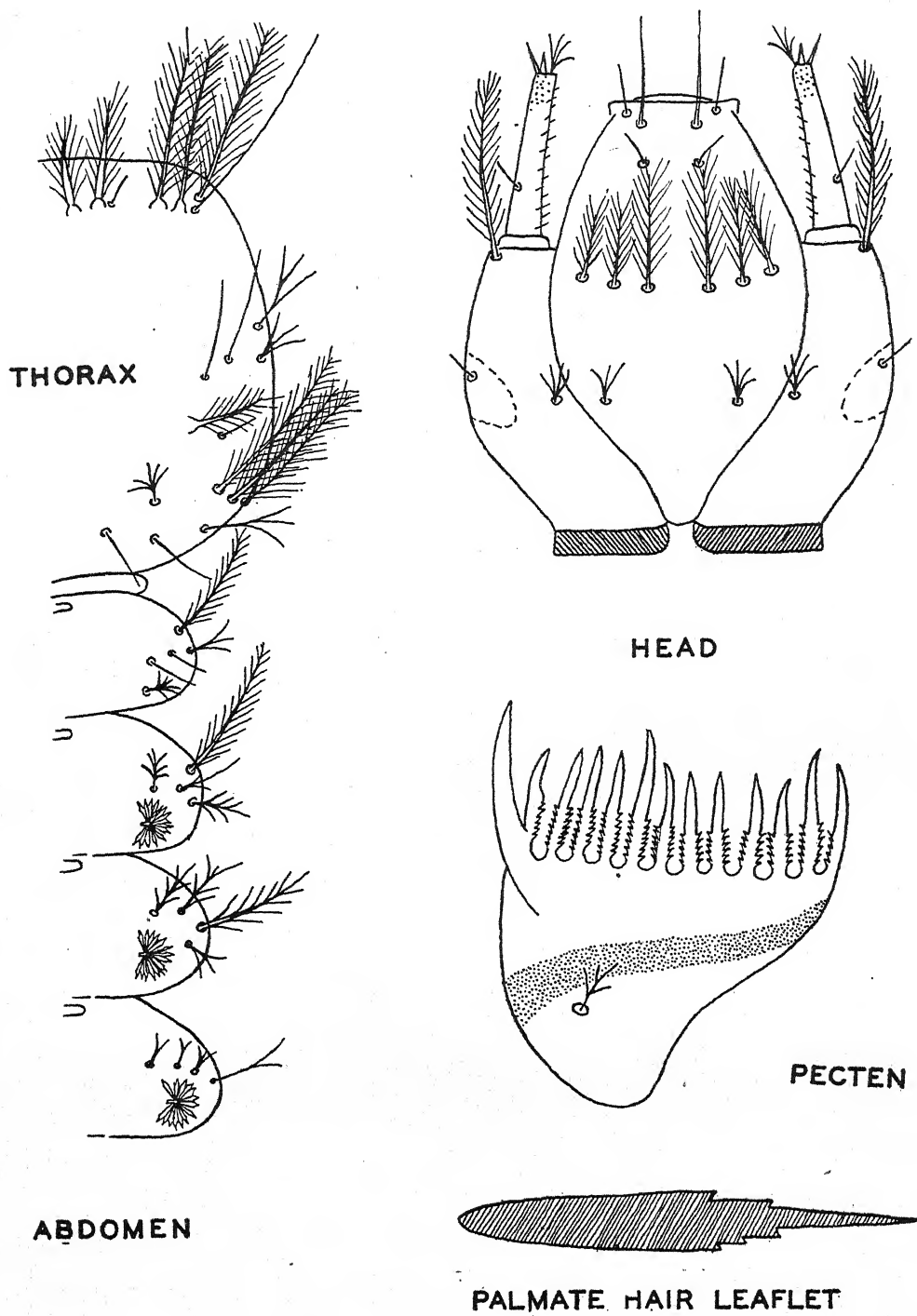


PLATE II

Morphology of larva of *Anopheles koliensis*



series D: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 5 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series E: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 7 females, 5 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 7, 1944 (Owen). Paratype series F: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 6 females, 4 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series G: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 4 females, 4 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 6, 1944 (Owen). Paratype series H: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 4 females reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 10, 1944 (Owen). Paratype series I: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 14, 1944 (Owen), and 9 females, 3 males reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 4, 1944 (Owen). Paratype series J: 1 female collected alive from tent, Nalimbu River, Guadalcanal Island, July 16, 1944 (Owen), and 5 females, 1 male reared from eggs deposited by that female in the laboratory, Koli Area, Guadalcanal Island, August 10, 1944 (Owen). Paratypes, 10 females collected resting in tent, Nalimbu River, Guadalcanal Island, July 13, 1944 (Owen). Paratypes, 4 females, 1 male reared from larvae collected in temporary pool at Metapona River Bridge, Guadalcanal Island, June 20, 1944 (Owen). Holotype, allotype and paratype series A, B, C, D, E, and F to be deposited in U. S. National Museum.

*Identification.*—This species is closely related to *Anopheles punctulatus* Dönitz and *Anopheles farauti* Laveran. Both males and females of *koliensis* can usually be separated from *punctulatus* by the absence of a small dark spot on the costal margin of the wing between the basal and median dark spots. The presence of a light patch of scales on the ventral side of the apical third of the labium in the female *koliensis* distinguishes it from the female of the above form. The larvae of this species are separated from *punctulatus* by having relatively short occipitals with three to four branches, enlarged shaft of prothoracic hair 1, well-developed palmate hairs on II, lateral hairs on IV bifurcate, and the structure of the pecten. As a rule the costal marking of the wing will separate both males and females of *koliensis* from *farauti*. The females of these two forms are also easily distinguished by the absence of light scales on the labium of *farauti*. The larvae of *koliensis* can be separated from *farauti* by having unbranched clypeal hairs, the basal tubercles of prothoracic hairs 1 and 2 separated, and the structure of the pecten. The species *koliensis* is closely related to another member of the *punctulatus* group which has a pale area on the ventral surface of the labium. This form is not known to occur east of New Guinea. It is usually classified as *Anopheles moluccensis*, Swg. and Swg. Whether these two forms are identical remains to be determined by future studies. The taxonomic relationships of the *punctulatus* group are obscure at the present. Rather than risk confusion by assigning the new material from the central Solomon Islands to *moluccensis* it was believed desirable to establish a new species.

Since *Anopheles koliensis* is morphologically in an intermediate position between *Anopheles punctulatus* and *Anopheles farauti*, the possibility that it is a hybrid of the two must be considered. Data which discredit this proposal are presented in a later section on experimental studies.

*Distribution.*—*Anopheles koliensis* is known to occur along the north coast of Guadalcanal Island from Koli Point to the vicinity of Aola Bay.

*Biology.*—The larvae of this species have been collected from temporary pools in the grassland and in the pools along the edge of the jungle. They prefer water exposed to sunlight rather than dense jungle conditions. They have always been associated with *farauti* and in one locality were collected from the same waters with



*farauti* and *punctulatus*. Information obtained on breeding habits of this species indicates that the larvae are relatively scarce during the dry season. No data have been obtained on breeding during the rainy season. The adults are strongly anthrophilic and are to be found resting during the day in native huts in greater numbers than any of the other local anophelines. Intensive studies were made in one locality for a period of several weeks on the relative abundance of adult anophelines found resting in tents occupied by personnel of a native labor group. Three anophelines were encountered in these observations, namely *koliensis*, *farauti*, and *punctulatus*. Five hundred adult females collected from these tents were checked for identity and of this number 90% were *koliensis*, 5.8% were *farauti* and 4.2% were *punctulatus*. Data on relative abundance of the larvae of these three forms in this area during the period of the studies show that the larvae of *koliensis* constituted approximately 10% of the total anophelines. The results of this study emphasize the fact that *koliensis* has a greater preference for entering human dwellings than the other anophelines commonly found in the area. Limited observations on flight habits of the females have shown that they become active about 9:00 PM and continue to fly until daylight. The period of greatest nocturnal activity was after midnight.

*Experimental Studies.*—The species has been repeatedly reared in the laboratory by obtaining eggs from gravid females captured in native dwellings. The average time required to develop from egg to imago under these conditions is eleven days. Adult females reared in this manner and confined in a cage were observed to feed readily on man.

The two cardinal characteristics which separate the adults of *koliensis* from *farauti* and *punctulatus*, namely, the costal markings of the wing and the light patch of scales on the ventral side of the labium, were studied for degree of variation. The individuals utilized in this study were the progeny of fifty wild females of *koliensis*. Each of these gravid females was isolated and her progeny reared separately. The parents were kept for comparison with their offspring. A total of five hundred progeny were obtained and of this number three hundred were females and two hundred were males. A summary of this experiment is as follows: (1) Each parent had the typical wing marking of *koliensis*. (2) The size of the labial light patch varied among the parents from a small group of scales to a larger patch which was visible from above at the apex. (3) Among the female offspring there were only three that possessed evidence of a black spot on the wing between the basal and median dark spots. This character appeared in the wing on only one side of the body. (4) There were twelve of the male offspring which had a small dark spot between the basal and median dark spots of the wing. As was observed among the females, this character appeared in only one of the wings. (5) The size of the labial patch among the three hundred females exhibited no greater range in variation than was observed among the parents. There was also no pronounced correlation between the size of this character in the parent and her respective offspring. (6) The results of this study combined with an examination of several hundred additional specimens of this form leads to the conclusion that these two adult characters are quite stable.

*Summary.*—A new anopheline was discovered on Guadalcanal Island and is described as *Anopheles koliensis*. Evidence derived from breeding experiments of a limited nature and from an examination of several hundred wild adults establish it as a true species. Studies on habits of adults lead to the conclusion that it is of primary importance in disease transmission.

# ANOPHELINE MOSQUITOES OF THE SOLOMON ISLANDS AND NEW HEBRIDES

JOHN N. BELKIN, Captain SnC, AUS, KENNETH L. KNIGHT, Lieutenant, H(S),  
USNR AND LLOYD E. ROZEBOOM, Lieutenant, H(S), USNR

## INTRODUCTION

Until less than three years ago, only two anopheline mosquitoes, *Anopheles punctulatus* and *A. punctulatus moluccensis*, had been reported as occurring in the Solomon Islands and New Hebrides. However, the presence of *A. punctulatus* was questionable as a state of confusion existed in regard to the exact taxonomic position of these species. Extensive collections during the past two years have revealed the existence of at least six species in this area. We feel that sufficient data have been accumulated to warrant a summary of the taxonomic status and the biology of these mosquitoes. Inasmuch as these species differ considerably in their habits, the presentation of this information should facilitate the determination of the relative importance of the disease transmitters as well as the formulation of control measures directed against the dangerous species.

The most intensive collections have been made on Espiritu Santo in the New Hebrides and on Guadalcanal in the lower Solomon Islands, and involved not only the rearing of adults from individual larvae taken from as many natural breeding places as possible, but also the rearing of progeny of isolated females, which had been captured in natural resting places and native dwellings. Such rearings enabled us to determine the extent of normal variation within "families," and to select the characters which remained constant and hence were of specific value. The distribution of the species in other islands of these groups is based upon material taken by a number of collectors.

## KEY TO SPECIES

### *Adult Females*

1. Costa without contrasting dark and light spots, palpi approximately one-third the length of the proboscis. . . . . *Bironella hollandi*  
Costa with contrasting dark and light spots, palpi as long as the proboscis. . . . . 2
2. Scutum with a vestiture of golden hairs, scales absent except on anterior promontory and near wing root; halteres white. . . . . 3  
Scutum with a vestiture of yellowish-white, broad, recumbent scales throughout; halteres dark-scaled on apex. . . . . 4
3. Apical third of labium yellow-scaled; fourth (morphological) segment of palpus with the basal dark ring covering approximately one-fifth of the segment  
*Anopheles lungae*  
Labium dark except for a small patch of bronzy yellow scales on the apical fifth or less; fourth segment of palpus with the basal dark ring covering approximately one-third of the segment. . . . . *A. solomonis*
4. Labium dark-scaled except for an inconspicuous pale ring at extreme apex, rarely with a few ventral pale scales on basal half. . . . . *A. farauti*

Received for publication, April 25, 1945.

- Pale scales present on apical third to half of labium. . . . . 5
5. Apical third to half of proboscis entirely pale except for a narrow subapical dark ring and scattered dark scales ventrally, subapical ring occasionally absent; usually a small dark sectoral spot on costa between the basal and median dark spots. . . . . *A. punctulatus*
- Apical third of proboscis with a ventral patch of pale scales which may be limited to a few scales or may be extended dorsally near apex to form an incomplete ring; usually no dark sectoral spot on costa between basal and median dark spots. . . . . *A. koliensis*

#### Adult Males

The characters used to separate the males of *Anopheles* are not entirely satisfactory; nevertheless, they will serve to distinguish the majority of specimens.

1. Costa without contrasting dark and light spots, palpi one-sixth of proboscis  
*Bironella hollandi*
- Costa with contrasting dark and light spots, palpi as long as proboscis. . . . . 2
2. Scutum with a vestiture of golden hairs; scales absent except on anterior promontory and near wing root; halteres white. . . . . 3
- Scutum with a vestiture of yellowish-white, broad, recumbent scales throughout; halteres black-scaled on apex. . . . . 4
3. Labium all dark-scaled except for apical light ring. . . . . *Anopheles lungae*
- Apical sixth to fifth of labium with a dorsal patch or incomplete ring of bronzy yellowish scales. . . . . *A. solomonis*
4. A small dark sectoral spot on costa between basal and median dark spots. . . . . 5
- No dark sectoral spot on costa between basal and medium dark spots. *A. koliensis*
5. Labium with at least two or three ventral light spots, frequently large patches of light scales. . . . . *A. punctulatus*
- Labium all dark except for apical light ring. . . . . *A. farauti*

#### Pupae

1. Paddle with fringe hairs on external margin only (Fig. 19); hair 5 of abdominal segment VI external to hair 10 on the posterior margin of the segment (Fig. 16); no oblique lateral margin to abdominal sternites.  
*Bironella hollandi*
- Paddle with at least some fringe hairs internal to the paddle hairs (Fig. 20); hair 5 of abdominal segment VI internal to hair 10 on the posterior margin of the segment; sternites of abdominal segments with distinct oblique lateral margins. . . . . 2
2. Paddle with a series of strong denticles on the external margin (Fig. 20). . . . 3
- Paddle without denticles, fringe consisting of fine hairs only (Fig. 19). . . . . 4
3. Lateral spine (hair 1) of abdominal segment V approximately equal in length to that of segment III; lateral spine of segment VII extremely slender and pale, usually branched or apically frayed (Fig. 26, B and C).  
*Anopheles lungae*
- Lateral spine of abdominal segment V approximately two to three times the length of that of segment III; lateral spine of segment VII stouter, darker, unbranched (Fig. 26, A). . . . . *A. solomonis*

4. Trumpet pale, at most contrasting but little with the cephalothorax; hair 8 of the cephalothorax over 105 microns in length; hair 10 of abdominal segment II with 12 or more branches; lateral spine of segment VII elongate, slender, rarely showing any fraying (Fig. 26, H). . . . . *A. punctulatus*  
Trumpet brightly pigmented from apex to basal notch; hair 8 of cephalothorax under 100 microns in length; hair 10 of abdominal segment II with fewer than 12 branches; lateral spine of segment VII, shorter, stout. . . . . 5
5. Lateral spine of abdominal segment VII nearly always frayed or branched, shaped as shown in Fig. 26, D and E; apex of male genitalia as in Fig. 17. . . . . *A. farauti*  
Lateral spine of abdominal segment VII rarely branched or frayed (Fig. 26, G, shows the type of branching which occurs occasionally), shaped as illustrated in Fig. 26, F; apex of male genitalia as shown in Fig. 18. . . . . *A. koliensis*

#### Fourth Instar Larvae

1. Hair 1 of mesothorax with stout shaft and many lateral branches; inner clypeals widely spaced, closer to outer clypeals than to each other; antennal hair minute. . . . . 2  
Hair 1 of mesothorax with the structure of a small palmate tuft; inner clypeals closely approximated, with contiguous tubercles; antennal hair large, plumose. . . . . *Bironella hollandi*
2. Prothoracic pleural group with one long hair branched; outer clypeals short, scarcely projecting beyond the clypeus or, if longer, then posterior clypeals 2-5-branched. . . . . 3  
Prothoracic pleural group with all long hairs single; outer clypeals extending at least half their length beyond the clypeus, posterior clypeals single, rarely double. . . . . 4
3. Outer clypeals extremely short, scarcely projecting beyond the clypeus; posterior clypeals extremely short, not reaching the tubercles of the anterior clypeals, usually single, rarely double; hair 1 of abdominal segment II with long, slender, unnotched leaflets; long metathoracic pleural hairs usually single, rarely one split into 2 branches. . . . . *Anopheles lungae*  
Outer clypeals a third to a half as long as the inner; posterior clypeals extending to or beyond the tubercles of the anterior clypeals, with 2-5 branches; hair 1 of abdominal segment II almost as large as those of segment III, with broad notched leaflets; metathoracic pleural group with one of the long hairs usually split into 2-3 branches. . . . . *A. solomonis*
4. Hair 1 of abdominal segment I a true palmate tuft, with broad, flattened leaflets; tubercles of prothoracic hairs 1 and 2 fused. . . . . *A. farauti*  
Hair 1 of abdominal segment I not resembling a palmate hair, but with narrow, hair-like branches; tubercles of prothoracic hairs 1 and 2 separate. . . . . 5
5. Outer occipitals usually with five or more branches (occasionally three to four); posterior clypeals short, not reaching the tubercles of the anterior clypeals; hair 2 of abdominal segments IV and V usually single or double; hair 6 of these segments usually double. . . . . *A. koliensis*  
Outer occipitals usually with 1-3 branches (rarely four); posterior clypeals usually extending to, or beyond, the bases of the anterior clypeals; hair 2



of abdominal segments IV and V usually triple; hair 6 of these segments usually triple. .... *A. punctulatus*

## CONSIDERATION OF SPECIES

*Bironella (Brugella) hollandi* Taylor

1934. *Bironella (Brugella) hollandi*, Taylor, Proc. Linn. Soc. N. S. W. 59: 229 (♂ and ♀).

**Type locality:** Kavieng, New Ireland. **Types:** Univ. of Sydney, Australia.

1944. *Bironella (walchi?)*. Belkin and Schlosser, Jour. Wash. Acad. Sci. 34: 268-273.

**ADULT FEMALE.**—A small, dark anopheline with unspotted wings and tarsi; palpi short. Length of wing 3-3.5 mm.

**Head:** White frontal tuft shorter than normal, lower setae barely reaching clypeus; vertical setae and long, erect, narrow, curved vertical scales white; erect occipital scales black, deeply forked and exceptionally long and slender, almost as long as frontal tuft setae. Antennae approximately two-thirds as long as proboscis; segments black except at base; torus dark brown; no scales on torus or first flagellar segment. Palpi usually slightly more than one-quarter length of proboscis, black-scaled. Labium black-scaled, slightly shorter than front femur. Labella light to dark brown. Buccopharynx without teeth.

**Thorax:** Scutal integument chocolate-brown; fossae, posterior extensions, and scutellum lighter; under proper illumination these areas, together with prescutellar space, and narrow median longitudinal scutal line, gray pollinose; scutellar disc usually dark, or entire scutellum often dark; scales entirely absent; very sparse vestiture of golden hairs; bristles dark. Halteres light basally, upper two-thirds of stem and knob dark; knob and part of stem with very short dark scales. Pleural integument variable in color, usually dark with lower mesepimera and lower sternopleura pale; scales entirely absent. Propleurals absent; spiraculars absent; lower sternopleurals 4-6 light hairs; upper sternopleurals 2 long dark bristles; prealars 3-5 light bristles; lower mesepimerals absent; subalar 1 dark bristle.

**Wing:** Vein scales, except on costal border, light gray, appearing white under proper illumination; fringe a slightly darker shade of gray; entire length of costa and vein 1 to slightly beyond middle of wing with scales much darker and denser giving appearance of black costal border in sharp contrast to rest of wing which appears light gray. Vein 2 approximately one and one-quarter times length of vein 2.1; vein 3 extending basad from cross-veins as a line of scales usually one-third of distance to wing base, just distad of cross-vein, usually a slight upward curvature; petiole of second posterior cell (vein 4 distad of cross-veins) approximately as long as vein 4.2, a downward curvature near its middle; vein 5.1 with downward curvature just distad of cross-vein; vein 6 with slight curvature in middle and at base; base of second posterior cell distinctly basad of second marginal cell; cross-vein at base of vein 2 clouded; cross-veins 2-3 and 3-4 in line; cross-vein 4-5 usually just basad of 2-3 and 3-4 and parallel to them.

**Legs:** Front femora slightly swollen, dark except for narrow central pale line on posterior surface from near base to apex, connected at base with a dorsal pale line which extends dorsally for approximately half the length of femur, anterior surface with a few scattered pale scales; front tibiae dark except for a narrow line of pale scales ventrally on posterior surface. Middle femora dark except for narrow pale line from base to within tenth of apex; middle tibiae dark except for narrow pale line from base to apex. Hind femora with entire anterior surface pale-scaled from base to apical fifth, posterior surface mottled with pale scales from base to apical fifth, narrow line of pale scales dorso-posteriorly extending halfway to apex; hind tibiae with narrow line of pale scales on anterior surface from basal sixth to apical fifth. posterior surface dark. Coxae and trochanters pale. All tarsi dark dorsally, lighter ventrally. Claws small, simple, and equal on all legs.

**Abdomen:** Dark brown, covered with moderately dense brown hairs on sternites and tergites, hairs more numerous and lighter on distal segments; no scales; cerci without scales, densely covered with light hairs.

**ADULT MALE.**—In general as in the female. White scaling on head more restricted; occipital scales fewer in number. Palpi usually slightly less than one-sixth the length of proboscis. Proboscis almost one-fifth longer than front femur. Thoracic vestiture even more restricted. Fifth segment of front tarsus shorter than fourth, cylindrical; claw simple. Sidepieces with very narrow dark scales.

**Genitalia** (Figs. 3 and 4): Sidepiece short, stout, about twice as long as broad; outer surface clothed with rather short hairs; a patch of long, narrow scales basally on dorsal surface. Basal lobe prominent, with a group of 9-15 stout spines at apex, 8 spines according to Taylor. A dense clump of long hairs arising proximally from ventral surface of basal lobe. Clasper long,



slender, bulbous at base and narrowing abruptly distally, sharply curved before apex; terminal spine short, rounded, and only slightly projecting beyond tip of clasper. Mesosome very long, slender; at the apex a pair of long, downwardly and laterally curving forked appendages, the branches of which are coarsely serrate. Claspette long, erect, the dorsal surface strongly convex medially, this convexity clothed with a dense patch of short, appressed, curved spines; apex with a stout digit-like process and two membranous appendages with expanded tips.

**PUPA.—Cephalothorax:** Trumpet (Fig. 23) darkly pigmented, scoop-shaped. Head shield distinctively shaped (Fig. 27), index of width to length 1:1.16 (range 1.00 to 1.35). External margin of palpal case evenly rounded (Fig. 27). Internal margin of median keel indistinct. Metanotal bar with a series of longitudinal wrinkles of which the median is heaviest, no definite lateral margins (Fig. 16).

**Abdomen** (Fig. 16): Hair 5 of segment VI external to hair 10.<sup>1</sup> Lateral spine (hair 1) of segment V five to ten times the length of that on III, generally twice the length of that of IV, and one-half to three-fourths the length of the lateral spines of VI and VII; lateral spines of segments IV–VII gradually tapered, with rounded apices, never branched or frayed (Fig. 25). Table 3 summarizes the number of branches possessed by the more important hairs.

**Paddle** (Fig. 19): Buttress only slightly developed. External border of midrib distinct to posterior one-fourth, internal border indistinct; no granulation of midrib or buttress. Fringe on external margin only, extending from upper one-fourth posteriorly to hair 12, consisting of long fine hairs. Hair 12 short, stout, straight, dark and sharply tapered. Hair 13 fine, approximately equal in length to 12, and pale.

**Genitalia:** Figs. 22 and 24 illustrate the male and female pupal genitalia.

**LARVA.—Head** (Fig. 8): Inner anterior clypeals long, single, closely approximated, the tubercles contiguous; outer clypeals single, one-third to one-half the length of the inner; posterior clypeals very small, single or double. Frontal and subantennal hairs long, plumose. Occipital hairs short; inner 2–4, outer 2–5-branched. Terminal hair of antenna long, plumose. Antennal hair 5–13-branched, situated one-third from base and reaching almost to or beyond apex of antenna.

**Thorax:** Prothoracic hair 1 without tubercle; stem short, slender, with 8–16 long, radiating branches; hair 2 with small tubercle, about twice the length of hair 1, with 12–15 branches (Fig. 9). Prothoracic pleural hair 9 long, split distally into 2–4 branches; hair 10 long, single; hair 11 small, 2–4-branched; hair 12 long, single (Fig. 10). Anterior mesothoracic pleural hairs (Fig. 11) long; hair 11 very small, single; hair 12 single, about one-fourth to one-third the length of the anterior pair. Anterior metathoracic pleural hairs (Fig. 12) long, single; hair 11 very small, single; hair 12 short, 2–4 branched. Hair 1 of meso- and metathorax with the structure of a small palmate tuft.

**Abdomen:** Palmate hairs present on segments I–VII; those on segment I somewhat reduced; leaflets long, narrow, edges smooth. Anterior tergal plates large, in width about two-thirds the distance between the palmate hairs; dorsum of segment VII almost entirely covered by tergal plate. Small posterior tergal plate present on segments III–VII. Hairs 6 and 7 of segments I and II long, plumose; hair 6 of segment III long, but with fewer branches; hair 6 of segments IV and V long, single. Postspiracular hair long, with about 5 branches; saddle hair long, single. Pecten with 6–8 short and 6–9 long spines.

**Egg** (Fig. 7). Broad anteriorly, tapering posteriorly; color black; approximately 0.55 mm. in length by 0.2 mm. in width (including floats). Floats large, almost as long as the egg; 15–20 float ridges. Frill with the shape of a broad collar at anterior end. Exochorion with fine reticulations forming an irregular, polygonal pattern.

**Taxonomic Discussion.**—The subgenus *Brugella* to which this species is assigned is characterized by short palpi, simple antennae in the male as well as the female, and by the absence of the basal arm and the presence of a clump of stout spines at the base of the sidepiece in the male genitalia. *B. hollandi* Taylor and *B. travestitus* (Brug) are the only species of the subgenus known in the adult stage at the present time. The original and subsequent descriptions of *B. travestitus* are too brief in their treatment of adult morphology and ornamentation to permit the separation of the two species in the female. The characters of the darker pleurae

<sup>1</sup> The position of hair 5 of abdominal segment VI in *Bironella* seems to offer excellent evidence that hair 9 of the genus *Anopheles* is actually hair 5, but shifted just internal to 10. Because of this, the nomenclature system for pupal hairs proposed by Rozeboom and Knight (1945, m. s.) and utilized here is being modified by changing hair 9 of segment VI to hair 5.

and differences in the curvature of veins used by Taylor to distinguish *B. hollandi* from *travestitus* are variable in the former species. *B. hollandi* and *travestitus* can be differentiated by the details in the structure of the claspette, and by the prominent basal lobe with its patch of long hairs and 8-15 stout apical spines in the former species. In *travestitus* there are only 4 long spines at the base of the sidepiece, and they apparently are not situated on a prominent lobe; furthermore, the group of long hairs seems to be absent (Swellengrebel and Rodenwaldt, 1932).

The larvae of *B. hollandi*, *travestitus*, *gracilis*, and *walchi* are characterized by the large plumose antennal hair and by the long external clypeals, which are at least a third as long as the inner clypeals. The known larvae of the other species of *Bironella* have a small antennal hair and very short outer clypeals, which are less than a fourth the length of the inner clypeals. Van Hell (1938) describes the long pleural hairs of *travestitus*, *gracilis*, and *walchi* as being single; in *hollandi* hair 9 of the prothoracic pleural group is split into 2-4 branches (Fig. 10). The outer clypeals of *walchi* are as long as the inner, while in *hollandi* they are one-third to one-half the inner. Prothoracic hair 1 of *walchi* is only 4-5-branched; in *hollandi* it is 8-16-branched. Taylor suggests the possibility that *hollandi* is the same as *walchi*, but this is not supported by the differences between the larvae. In *gracilis* the outer clypeals are 2-5-branched or even plumose; in *hollandi* they are single. The larva of *travestitus* seems to be very similar to that of *hollandi*, except for the outer anterior pleural hair (hair 9).

*Distribution*.—GUADALCANAL. Northwest and north central coast: Marovovo, Visale, Tenamba, Aruligo, Doma Cove, Segilau, Bunina, Tassafaronga, Bonegi, Mamara, Poha, Kokumbona, Matanikau, Kukum, Lunga, Teneru, Koli; southwest coast: West Cape. RUSSELL ISLANDS. Banika (W. G. Downs). NEW GEORGIA GROUP. Munda (J. G. Franclemont). BOUGAINVILLE. Empress Augusta Bay (A. B. Gurney).

*Biology*.—On the northwest coast of Guadalcanal *Bironella hollandi* is the commonest anopheline breeding in undisturbed, natural, permanent or semi-permanent bodies of water, such as streams, swamps, and dense coastal lagoons at the mouths of rivers. Bomb craters, "fox holes," road ruts, hog wallows, and even artificial containers in at least partial shade are sometimes utilized as breeding places. The larvae occur most frequently in shaded situations, and have been taken in places so dark that they could not be seen in a white dipper. Although they have been collected in very exposed situations, such as small canebrake swamps in grasslands, even here they are usually found deep in the vegetation where there is very little light. The dry season of the year forces the species into quite open situations such as pools in beds of dry streams. During the rainy season, the streams are flushed, the swamps expand and larvae are more difficult to find, although the species continues to breed in large numbers, as collections through every month of the year indicate. The larvae tolerate water with a high organic content such as is found in dense canebrake swamps, but occur more frequently in cool, clear water in vegetation or flottage.

The anophelines most frequently associated with this species are *A. lungae* and *solomonis*. In more exposed situations it is frequently associated with *farauti* and very rarely with *punctulatus*. A species of *Dixa*, several species of *Culex* (*Lophoceratomyia*), *Uranotaenia argyrotarsis* Leic. and *U. tibialis* Taylor are frequently found with *B. hollandi*.

There are two color phases of the larvae of this species, light yellowish-brown and black. Both phases may be recognized macroscopically from other local anophelines by the light coloration of the entire fourth abdominal segment which is in strong contrast with the uniformly darker coloration of the rest of the abdomen; the other anophelines may have light markings on the abdomen, but these are not restricted to the fourth segment. The pupae are smaller and wider than those of the other anophelines and have shorter and more widely-flared trumpets. The eggs float on the surface of the water like those of other anophelines, but can be distinguished from local species by the meniscus they form on the surface, which is straight instead of being cross-shaped.

Larvae brought from the field are rather difficult to rear and take approximately ten days to two weeks from the first instar to pupation; the pupal stage lasts two to three days. The adults are very fragile and are difficult to keep alive in captivity. They exhibit the normal anopheline resting position, but because of their extremely thin bodies and excessively elongate legs, they resemble small crane flies. Nothing is known about their habits beyond the fact that the females do not feed on man. All the adults in collections have been reared. Searches for resting places have been made without success in the vicinity of breeding places.

*Anopheles (Myzomyia) solomonis*, n. sp.

**ADULT FEMALE.**—A medium-sized, yellowish-gray, speckled anopheline; apical sixth to fifth of labium with dorsal patch or ring of yellow scales. Length of wing, 4 mm.

**Head:** Conspicuous white frontal tuft; vertical setae white, followed by one or two rows of white narrow hair-like scales; white scales in middle of vertex forming a wide spot narrowed in center; posteriorly the light scales with a yellow tinge; the rest of the vertex and all occipital scales dark. Antennae with a few minute white scales on torus, dense white scaling on first flagellar segment. Palpi as long as proboscis, ornamented as shown in Fig. 2; second morphological segment black with very narrow ring of white scales; third segment black with very narrow incomplete ring of white scales; fourth segment black-scaled basally for a third of its length, remainder white in center and yellowish basally and apically; fifth segment black-scaled for a third of its length, remainder yellowish. Ratio of fourth segment to third segment 1:1.7. Labium (Fig. 2) dark-scaled except for a dorsal patch of yellowish bronzy scales which may extend ventrally, and an apical light ring; differentiation between the coloration not distinct. Labella dull yellow. Buccopharyngeal armature (Fig. 6) of several broad central teeth forming a single row; teeth of similar character, separated by intervals; apices of teeth deeply serrated, bases without bullae or lateral spines.

**Thorax:** Scutal integument light yellow-brown with gray pollinose longitudinal lines; dark brown eye spots in front of and behind scutal angle; prescutellar space and disc of scutellum dark brown. White scales on anterior promontory rather short and sparse, central scales elongate, lateral broader; 15–25 black scales on humeral angles below lateral tufts. Rest of scutum devoid of scales; vestiture consisting of numerous golden hairs of varying length. Scutal and scutellar bristles light in color. Prothoracic lobes with a large patch of short black scales on upper part. Pleura without scales, integument dark gray with usual darker lines and spots. Spiracular bristles absent; propleurals 6 stout dark bristles; lower sternopleurals several golden hairs; upper sternopleurals 2–3 dark bristles and varying number of light hairs; prealars 4–8 golden hairs; subalars 5–10 golden hairs; lower mesepimerals absent. Halteres white with white scales on shaft and knob.

**Wing** (Fig. 1): On the costal border three dark and three light humeral spots, third light humeral spot sometimes obliterated; basal dark spot solid on costa, subcosta and vein 1, usually narrowed on 1; sectoral spot variable in size; accessory sectoral spot usually reaching costa; dark sectoral spot narrowed on subcosta, frequently absent on vein 1; median dark spot very large, wedge-shaped, extending to base of vein 2, on vein 1 dark scaling may be interrupted by light scales distally, small dark spot thus formed migrating occasionally into subcostal spot; subcostal spot large; preapical dark spot extremely variable, may be large and solid on both costa and vein 1 or on costa alone, or broken into two to four small spots on these veins, or represented by one very small spot on each one of these veins; preapical spot sometimes reduced;

apical dark spot solid, including veins 1 and 2.1; apical spot smaller than apical dark spot. Wing veins light-scaled with the following dark spots: vein 2 with large dark spot involved in median dark spot and one to two small dark spots distad; vein 2.1 with 2 to 3 basal small dark spots and one large spot involved in apical dark spot, apex of vein light-scaled; vein 2.2 with 5 to 7 small dark spots, evenly spaced, vein dark at apex; vein 3 with 5-8 small dark spots, apex of vein light; vein 4 with 3 to 7 dark spots, large one usually at bifurcation; veins 4.1 and 4.2 each with 2 to 4 dark spots, large one usually at base continuous with apical dark spot of vein 4; vein 5 with 3-6 rather evenly spaced small dark spots; vein 5.1 and 5.2 each with 3-5 small dark spots; vein 6 with 4 to 7 small dark spots. Fringe white except for the following dark spots: large spot between veins 2.1 and 2.2; small spots below apex of veins 3 (frequently absent), 4.1, 4.2, and 5.1; large spot at apex of vein 6. Light wing scales yellow, dark scales black. Venation and cross-veins normal.

**Legs:** Front femora swollen in basal half, speckled with light scales; middle and hind femora and all tibiae with numerous, rather evenly spaced pale spots. First segment of all tarsi with numerous light spots and light apex. Second, third, and fourth segments of front tarsi with broad basal and apical light bands; second and third segments often with additional light spots in center; fifth segment dark except at apex. Second, third, and fourth segments of middle tarsi with narrow apical light bands, occasionally with light spots centrally; fifth segment dark except at extreme apex. Second segment of hind tarsi with narrow apical light band and usually one to three small light spots centrally; third segment with narrow apical light band and occasionally a few light scales centrally; fourth segment with narrow apical light band; fifth segment all dark. A few light scales basally on middle and hind tarsal segments, visible under high magnification. Extent of light scaling on legs very variable. Light scales on legs yellowish.

**Abdomen:** Integument dark gray. Devoid of scales on tergites and sternites I-VII, instead vestiture of narrow golden hairs similar to those on scutum. Hairs more numerous on posterior segments, grading into very narrow curved scales on tergite and sternite VIII. Cerci with narrow black scales.

**ADULT MALE.**—In general as in the female. First flagellar segment with tuft of narrow white scales. Second palpal segment with light scales dorsally in center; articulation between second and third segments light-colored, third palpal segment with narrow apical ring of yellow scales and patch of yellow scales dorsally in center; fourth and fifth segments yellow with narrow basal dark rings which extend externally to near apex of segments. Labium dark except for dorsal patch on incomplete ring of bronzy yellowish scales on apical sixth to fifth. Abdomen as in female except for numerous yellow scales on eighth tergite. Sidepieces densely covered with yellow scales ventrally (morphologically) and black scales laterally and dorsally.

**Genitalia:** Indistinguishable from those of *A. lungae*.

**PUPA.** The pupa possesses a brownish-yellow color which persists to a distinctive degree even in specimens reared completely through in the laboratory. This is in marked contrast to the *punctulatus* series, the members of which become nearly devoid of pigment in material reared from eggs. Tables 2 and 3 summarize the data of diagnostic value for this species.

**Cephalothorax:** Trumpet dark brownish-yellow. Index of width to length of head shield 1:0.98 (range 0.93-1.06). External margin of palpal case rounded, sometimes with an angulate point at the center of the curve. Internal margin of median keel sharply outlined. Metanotum with a distinct, smooth median bar bounded laterally by a crease which is incomplete posteriorly. Chaetotaxy similar to that of *B. hollandi* except that hair 11 is generally slightly anterior to a line drawn between 10 and 12.

**Abdomen:** Chaetotaxy generally similar to that shown for *B. hollandi* (Fig. 16) except for the following: sternites with strong oblique lateral margins. On segment I, hair 6 generally external to hair 9, occasionally in line with it; on segment III, hair 6 well anterior to a transverse line through hair 4, hair 18 absent; on segment IV, hair 6 well anterior to a transverse line through hair 5, on segment V, hair 5 nearer to hair 4 than to 8, much shorter than 8 in length; on segment VI, hair 2 on or very near the posterior margin of the segment, hair 5 on the posterior margin internal to 10, shorter than 10; on segment VII, hair 2 on the posterior margin of the segment, hair 5 well anterior to a transverse line through hair 4; on segment VIII, hair 8 very near to a transverse line through the lateral spine (hair 1). Lateral spine of segment V approximately two to three times the length of that of segment III; lateral spines of segments VI-VII (Fig. 26, A) slender, elongate, acutely tapered and only very rarely with branches or fraying, pigmented to about the same extent as the segments.

**Paddle** (Fig. 20): External border with stout, bluntly tapered denticles, which are pigmented to the same degree as the paddles. These denticles begin at upper one-fourth as minute teeth internal to the border, increasing in size posteriorly and extending to and along the external margin to the postero-lateral corner, the last few denticles being more acutely pointed



than the others; a fringe of long, pale hairs begins where the denticles terminate and extends mesally and forward along the inner margin of the paddle to the anterior one-fourth. External border of midrib obscure, internal border absent or very faint, granulations scanty or absent. Buttress with minute, scattered denticles and generally with areas of granulations. Hair 12 slender, pale, straight or curved. This hair is much in contrast to that of the *punctulatus* series where it is noticeably stouter and longer.

**Genitalia:** In male (Fig. 17), more heavily pigmented than any other part of the abdomen; the lateral margins of the genital lobes are nearly parallel from base to apical one-fourth, apex of lobes shaped as figured. Female genitalia similar in form to that illustrated for *farauti* (Fig. 21).

**LARVA.—Head:** Inner clypeal hairs very long, with many minute lateral branches; outer clypeals a third to a half the length of the inner, sometimes single but usually frayed; the distance between the outer and inner hairs one-fourth to one-third that between the inner hairs; posterior clypeals 2-5-branched and long enough to reach the tubercles of the anterior clypeals. Frontal and subantennal hairs feathered. Antennae with long spines on inner surface; antennal hair minute, situated one-third the distance from the base; terminal hair longer than sabers, 4-8-branched. Occipital hairs short; inner 2-5-branched, outer 2-6-branched.

**Thorax:** Tubercles, of prothoracic hairs 1 and 2 large, heavy, separated from one another; hair 1 with heavy shaft and many long radiating branches; hair 2 about twice the length of hair 1. Prothoracic pleural hair 9 (Fig. 13) long, with 5-10 branches; hair 10 long, single; hair 11 about one-fourth the long hairs, single or double; hair 12 long, single. Anterior pair of mesothoracic pleural group long, single, occasionally one hair double; hair 11 minute, single; hair 12 short, 2-4-branched. Outer anterior metathoracic pleural hair (9) split into 2-3 branches, inner hair long, usually single, sometimes double; hair 11 minute; hair 12 short, 2-4-branched (Fig. 14).

**Abdomen:** Hair 1 on segment I very small, with narrow, hair-like branches. Hair 1 on segment II almost full-sized, with broad, notched leaflets. Palmate hairs large on segments III-VI, smaller on segment VII, heavily pigmented on segments II-VII; leaflets very broad, abruptly narrowed, with deep indentations, into a short terminal filament that is about one-fourth as long as the main shaft. Hair 6 of segments IV and V 2-4 branched. Tergal plates darkly pigmented, small, half or less the distance between the palmate hairs except on segment VIII where it is large. Hair 13 of Segment V very large, with 7-13 branches. Pecten with 4-5 long and 6-10 short spines. Saddle hair long, single.

**TYPES.—Holotype** ♀ with larval and pupal skins, Poha River tributary, 3 miles south of the coast, Guadalcanal, 10 Sept. 1944 (L. E. Rozeboom). **Allotype** ♂ with larval and pupal skins, Bonegi River tributary, 2½ miles south of the coast, elevation 1500 feet, Guadalcanal, 21 July 1944 (L. J. Lipovsky). **Paratypes** (29 ♂, 41 ♀): 1 ♂ with pupal skin, Matanikau River tributary, Guadalcanal, 27 Nov. 1943 (J. N. Belkin); 1 ♀ with larval and pupal skins, Matanikau River tributary, Guadalcanal, 8 Feb. 1944 (J. N. Belkin); 1 ♀, Burns Creek, Lunga District, Guadalcanal, 25 May 1944 (J. N. Belkin); 2 ♀, 1 ♂ with larval and pupal skins, Bonegi River tributary, 2½ miles south of the coast, elevation 1500 feet, Guadalcanal, 21 July 1944 (L. J. Lipovsky et al.); 1 ♂ with larval and pupal skins, Matanikau River tributary, Guadalcanal, 6 Aug. 1944 (J. N. Belkin); 1 ♂, 2 ♀, Kokumbona River, 4 miles south of coast, Guadalcanal, 24 Aug. 1944 (M. Cohen, H. Sexauer, et al.); 1 ♂, 2 ♀ with larval and pupal skins, 2 ♂, 3 ♀ reared from pupae, Poha River tributary, 3 miles south of coast, Guadalcanal, 9-26 Sept. 1944 (L. E. Rozeboom, J. N. Belkin, J. Laffoon, et al.); 22 ♂, 30 ♀ with larval and pupal skins, progeny from gravid female collected resting on tree trunk, Sprague Swamp, Bunina, Guadalcanal, 13 Nov. 1944 (L. J. Lipovsky, M. Cohen, A. W. Barnes). **Holotype** and **allotype** to be deposited in U. S. National Museum. **Paratypes** to be deposited in the collections of the University of Sydney, Cornell University, Johns Hopkins School of Hygiene and Public Health.

**Taxonomic Discussion.** *A. solomonis* and *lungae* form a distinct complex and perhaps should be recognized as a separate series under the group *Neomyzomyia*. They fall into this group on the basis of buccopharyngeal armature (Figs. 5, 6), propleural hairs, presence of scaling on the pronotal lobes, and speckled legs in the adults. Other species of this group in the Oriental and Australasian regions having the scutal ornamentation restricted to the anterior promontory, entirely white halteres, speckled legs without a broad white band on the hind legs at the junction of the tibia and first tarsal segment are *A. tessellatus*, *longirostris*, *longirostris annulata*, and presumably *tessellatus orientalis*. *A. solomonis* and *lungae* differ from



all of these in having a very narrow ring of white scales at the apex of third palpal segment instead of a broad light band, covering usually about a half of this segment.

The larval pleural hairs show branching which is inconsistent with the definition of the group *Neomyzomyia*, but which is found in three other forms usually referred to this group: *A. amictus*, *amictus hilli*, and *novaguinensis*. The latter species all have one long hair of the mesopleural group at least two-branched, and one long hair of the metapleural group with five or more branches. In *A. solomonis* and *lungae*, the long mesopleurals are usually single, and both long metapleurals may be single (usually in *lungae*, rarely in *solomonis*), or at most with 2-3 branches (occasionally in *lungae*, usually in *solomonis*).

The adults of *A. novaguinensis*, *amictus* and *amictus hilli* possess conspicuous scales on the disc of the scutum which immediately separate them from *solomonis* and *lungae*.

Adult females of *A. solomonis* are separated from *lungae* by the restricted yellow coloration on the apical fifth or less of the labium, the proportionately shorter third palpal segment, the greater extent of the dark coloration on the base of this segment, the generally darker coloration of the legs, and differences in the buccopharyngeal armature. All males of *solomonis* examined showed some pale scales on the labium, a character which is very rarely present in *lungae*. The larvae of the two species are separated on the size of the outer clypeals, the size and branching of the posterior clypeals, and the structure of the palmate hair on abdominal segment II.

In a personal communication to one of the authors (JNB), J. G. Franclemont reports finding a form similar if not identical with *A. solomonis* on New Georgia Island. The only obvious difference noted in the brief description available is in the more extensive yellowish coloration of the proboscis which may cover the apical third.

*Distribution*.—GUADALCANAL. Northwest coast: Bunina, Tassafaronga, Bonegi, Poha, Kokumbona, Matanikau, Lunga. ? NEW GEORGIA. Munda (J. G. Franclemont).

*Biology*.—The larvae of *A. solomonis* have been collected most frequently in small tributary streams of the larger rivers at a distance of one to four miles into the coral foothills of the northwest coast of Guadalcanal at elevations to 1500 feet. They were found in pot holes in coral stream beds, in coral depressions above the stream beds, along the margins of the streams, and in the blocked mouths of the tributary streams where they are probably flushed from breeding places farther up the streams. One collection was made in a taro swamp with a depth of water of only one inch. The water in the breeding places may be clear and running, but is frequently stagnant, with a high organic content, and bluish in color. Practically all the collections have been made in deeply shaded areas in flottage of sticks or in leaves along the margins of the pools. The larvae are difficult to locate and are very scarce. They have the same habits as *lungae* of crawling up on leaves and banks out of the water. The pupae also work their way out of the water, as do those of *lungae*. The species may be collected throughout the year but is less difficult to collect during the dry months.

*A. solomonis* is frequently found where other anophelines are absent, but it has also been collected in association with *A. lungae*, *B. hollandi*, and once with *A. farauti* and *punctulatus*.

The larvae and pupae resemble macroscopically those of *A. lungae* in the shape of the body and coloration and cannot be separated from this species in the field.

From laboratory rearings, the length of life cycle appears to be the same as for *A. lungae*. All the eggs do not hatch at once and the larval stage lasts from 10 days to 2 weeks or longer, and the pupal stage from two to three days.

Little is known concerning the habits of the adults. Both males and females have been found in very small numbers resting in the daytime in the company of *A. lungae* on the buttresses and trunks of large trees in swampy jungle areas.

*Anopheles (Myzomyia) lungae* Belkin and Schlosser

1944. *Anopheles (Myzomyia) lungae* Belkin and Schlosser, Jour. Wash. Acad. Sci. 34: 268 (♂ and ♀). Type locality: Guadalcanal Island. Types: U. S. National Museum.

1944. *Anopheles* sp., Knight, Bohart and Bohart. Keys to the mosquitoes of the Australasian Region. Nat. Res. Council, Wash., p. 10.

ADULT FEMALE.—A medium-sized, yellowish, speckled anopheline; apical third of labium yellow-scaled. Length of wing 4-4.5 mm.

Palpi as long as proboscis; second and third morphological segments black with narrow ring of white scales apically, in 5 per cent of specimens studied (80), dorsocentral patch of dark bronzy scales also present on these segments; fourth segment black-scaled basally for approximately one-fifth its length, remainder white except frequently yellowish basally and apically; fifth segment black-scaled for approximately one-fifth of its length, remainder yellowish; ratio of fourth segment to third segment 1:1.35. Labium dark basally; apical third golden-yellow-scaled except usually a preapical dark ring; boundary between black and yellow sharply marked; in less than 5 per cent of specimens examined a few light scales basad of light apical area. Labella dull yellow. Buccopharyngeal armature (Fig. 5) of several teeth forming a single row; teeth of similar character, separated by intervals; apices of teeth serrated, bases with bullae but without lateral spines. Scutum without scales except on anterior promontory and in front of wing roots, vestiture of numerous golden hairs of varying length. Scutal integument light yellowish-brown; eye spots and dark areas as in *solomonis*. Prothoracic lobes with patch of black scales dorsally. Pleura without scales. Lower mesepimerals absent; propleurals 6 stout dark bristles; other pleural bristles and hairs as in *solomonis*. Halteres white with white scales on shaft and knob. Wing in general as described for *solomonis*. Legs as in *solomonis*, except that on front tarsi fifth segment usually with basal and apical light bands, fourth segment frequently with light spots in center dorsally; light coloration of front legs more extensive than in *solomonis*. Abdomen devoid of scales on tergites and sternites I-VII; vestiture of golden hairs of varying length; hairs more numerous on posterior segments, grading into narrow curved scales on apical border of segment VIII. Cerci with narrow yellow scales at apex, dark scales at base and sides.

ADULT MALE.—In general as in the female. Second morphological palpal segment usually with few white scales at apex and patch of yellow scales dorsocentrally; third segment with narrow apical yellow ring and patch of yellow scales dorsocentrally; fourth and fifth segments yellow with usually narrow basal dark rings; extent of light coloration variable. Abdomen and sidepieces as in *A. solomonis*. The male genitalia have been described and figured by Belkin and Schlosser. Some of the leaflets of the mesosome are serrate.

PUPA.—Very similar to the pupa of *A. solomonis* except for the following: lateral spines of abdominal segments IV-V each approximately equal in length to that of segment III; lateral spines of segments VI and VII (Fig. 26, B and C) extremely slender and pale, appearing to be less pigmented than the segments, frequently branched or apically frayed; the lateral spine of segment VII possessing one or more branches in over 80 per cent of the specimens examined. Tables 2 and 3 summarize the data of diagnostic value for the species.

LARVA.—Inner anterior clypeal hairs widely spaced, rather short, frayed; outer clypeals very short, extending at most only slightly beyond the clypeus; posterior clypeals single, rarely double, and very short, not reaching the tubercles of the anterior clypeals. Inner and outer occipitals short, 2-4-branched. Tubercles of prothoracic hairs 1 and 2 heavy, separated from one another; hair 1 with stout shaft and long radiating branches. Outer anterior hair (9) of prothoracic pleural group (Fig. 15) split into 3-6 (rarely 2) branches; anterior hairs of meso- and metathoracic pleural groups long, single.

Hair 1 of abdominal segment I with narrow, leaf-like branches. Hair 1 of abdominal segment 2 with long slender, unnotched leaflets. Palmate hairs large on segments III-VI, smaller on segment VII. Hair 6 of segments IV and V sometimes 1-2-, usually 3-branched.

*Distribution*.—GUADALCANAL. Northwest and north coast: Tenamba, Aruligo, Doma Cove, Segilau, Bunina, Umasami, Tassafaronga, Bonegi, Mamara, Poha, Kokumbonā, Matanikau, Kukum, Lunga, Tenaru, Koli, Tetere. NEW GEORGIA. Munda (J. G. Franclemont). BOUGAINVILLE. Empress Augusta Bay (A. B. Gurney).

*Biology*.—"The larvae of this species are normally found in the jungle in seepage areas, along the margins of streams, pot holes in stream beds, rock holes, dense jungle swamps, and temporary pools. The species has a decided preference for shade in its breeding places" (Belkin and Schlosser, 1944). Other breeding places noted are "fox holes," hog wallows, old steel helmets, dense canebrake swamps, and coastal lagoons at mouths of streams. The water in some breeding places may have a very high organic content, but usually is clear and cool. The larvae at times become quite numerous, but are hard to collect because of their habit of resting in very shallow water on the margins of the breeding places. They have been observed to crawl out of water and rest on the bank or on dead leaves floating on the water. The pupae also leave the water and in the laboratory normally rest on the sides of the rearing vessels, sometimes half an inch or more above the surface film. A film of water completely surrounds them, but does not connect with the surface of the water in the container. Usually emergence takes place from pupae resting above the surface of the water.

*A. lungae* increases in numbers during the rainy season when the breeding places become more extensive. The aquatic stages are even flushed from the hills into breeding areas in the coastal plain. On two occasions larvae and pupae were observed floating in a strong current in a small stream draining a large canebrake swamp. Several hundred larvae and pupae were collected in an hour's time among the debris held in place in the middle of the creek by an obstruction.

*A. lungae* has been collected commonly in association with *B. hollandi* and *A. solomonis*, rarely with *farauti*, and only once with *punctulatus*.

The larvae of *A. lungae* are easily distinguished in the field, except from *solomonis*, by the relatively broader abdominal segments, the transparency of the body and clear yellow pigmentation. The pale larvae of the other species have a much more opaque coloration (*A. punctulatus* and *farauti*) or are very narrow (*B. hollandi*).

A large number of rearings from eggs have shown some unusual characteristics of this species. All eggs do not hatch at once, even though they may be floating on the surface of the water. Some hatch three to four days after oviposition, others may not hatch for two weeks or longer. We have obtained adults from a batch of eggs before all first instar larvae appeared. The eggs are also capable of withstanding a considerable amount of drying. This seems to be an adaptation to breeding places which are periodically flushed. The larval development, once started, takes about ten days; the pupal stage usually lasts two to three days.

The diurnal resting places of the adults are easily located in swampy jungle areas where the species breeds in abundance. Males and unfed, blooded or gravid females are found together resting on the trunks and buttresses of various species of large trees growing in such areas. On several occasions they have been observed to rest also at the bases of various herbaceous plants. In uncontrolled areas, two or three hundred adults have been occasionally collected from a single tree. They

usually rest a foot or less above the ground where the humidity is very high and where there is very little light, but they have also been frequently collected resting as high as six feet above the ground on well-illuminated portions of the trunks. In some areas a third or more of the blooded or gravid females have been caught with Heleids of the genus *Culicoides* attached to their abdomens. In the daytime this species exhibits very little activity, never attempting to bite the collector, even though the density of the mosquitoes may be very high. When disturbed they will fly out a few inches or feet and again come to rest. The resting position is at an angle of approximately 60 degrees to the surface.

Nocturnal activity during the rainy season usually starts around 6:30 PM and by 7:00 or 7:30 PM all the adults have left their resting places. In the morning they have been observed to return to their roosts between 6:00 and 6:30 AM. Biting records and collections have been made at night in areas with a very high density of this species and a rather low density of *A. farauti*. *A. lungae* formed only 2 per cent of the total anopheline catch, while *farauti* comprised the remaining 98 per cent. In routine night catches near human dwellings conducted for a period of over a year in the Lunga area of Guadalcanal where this species is common, *lungae* accounted for less than 2 per cent of the total anopheline collections. No specimens of this species have to date been taken in native villages. Microscopic examination of the blood from females collected on tree trunks within a half mile of human dwellings revealed that approximately 70 per cent of the blood meals are nonmammalian in origin, the red blood cells being nucleated. There is no conclusive evidence at the present time as to the origin of the mammalian blood found in the stomachs of these mosquitoes. Precipitin tests, made on a small number of stomach contents, were inconclusive. It is felt that probably the majority of such blood is not of human origin as in addition to a variety of birds, a number of species of bats, a marsupial, many pigs, and occasionally cattle are present in the areas where *lungae* is abundant, and, as pointed out above, even with a high density of this species, it is not attracted to humans. It is possible that during the rainy season when the species is flushed into the coastal plain, relatively more available human hosts and scarcer natural hosts may induce *lungae* to feed on man. The experience on Guadalcanal during the last rainy season would seem to indicate that this takes place only locally.

*Anopheles (Myzomyia) punctulatus* Doenitz

1901. *Anopheles punctulatus* Doenitz, Insekten Boerse. 18: 36 (♂ and ♀). Type locality: New Guinea; Bismarck Archipelago. Types: unknown.

ADULT FEMALE.—A medium-sized, speckled anopheline, scales present on scutum, apical half of labium light. Size medium, length of wing 3-4 mm.

Head: Usual ornamentation on front, vertex and occiput. Antennae normal, with minute white scales on torus; patch of light scales on first flagellar segment. Palpi as long as proboscis; second morphological segment with narrow apical light ring, remainder usually black; third segment usually with narrow apical light ring, a broad subapical light band, separated by narrow dark ring; occasionally pale markings fused to form a very broad apical pale area; fourth and fifth segments broadly light with narrow basal black rings. Apical third to half of labium entirely pale except for a narrow subapical dark ring and scattered dark scales ventrally; subapical ring occasionally absent. Labella light. Buccopharyngeal armature of several teeth forming a single row; teeth of similar character, separated by intervals; apices of teeth deeply serrated; base of each tooth with bulla and a stout spine on each side.

Thorax: Scutum light yellow-brown to dark brown, darker eye spots anterior to scutal angle and dark prescutellar space. Anterior promontory with sparse elongate erect white scales in center, somewhat shorter, broader scales in lateral tuft, light above, dark below. Remainder of scutum with vestiture of broad, recumbent, yellowish-white scales and golden hairs; scales



somewhat longer in front and above wing base. Scutellum with disc dark; a few whitish scales, smaller than on scutum. Scutal and scutellar bristles golden brown. Prothoracic lobes dark with broad erect black scales dorsally. Pleural integument dark brown with usual light areas. Spiracular bristles absent; propleurals 2-3, rarely 1 or 4-6; lower sternopleurals usually 4 light hairs; upper sternopleurals 5-7 hairs; prealars a group of 4-6 light hairs; subalars a group of 4-10 light hairs; lower mesepimerals absent. Patch of 6-7 broad recumbent light scales on upper sternopleura, similar patch of 5-6 scales on lower sternopleura; rest of pleura bare. Halteres light at base, dark-scaled on knob.

*Wing*: As figured for *A. p. punctulatus* by Ross and Roberts (1943) but extremely variable. Costal border with four large dark spots; small dark sectoral spot usually present on costa between basal and median dark spots; small dark spots on vein 1 below median dark spot may coalesce, also small dark spots on vein 1 below preapical dark spot, giving much darker appearance to wing. Veins 2-6 with numerous small dark spots. Light scales yellowish-white. Fringe dark, light spots usually at apices of all veins.

TABLE 1.—Summary of diagnostic measurement\* data for pupae of *punctulatus* series

Species	Hair 8 of Cephalothorax Length				Hair 5 of Ab.† II Length			
	Range	Ave.	% under 105 mic.	% 105 mic. or over	Range	Ave.	% under 159 mic.	% 159 mic. or over
<i>punctulatus</i> .	95-166	111	2.3	97.7	119-238	181	2.9	97.1
<i>koliensis</i> ....	51-88	69	100.0	0.0	74-153	124	100.0	0.0
<i>farauti</i> .....	68-116	82	97.4	2.6	98-164	150	99.4	0.6
Species	Hair 1 of Ab. IV Length				Hair 1 of Ab. VI Length			
	Range	Ave.	% under 27 mic.	% 27 mic. or over	Range	Ave.	% under 104 mic.	% 104 mic. or over
<i>punctulatus</i> .	10-34	17	99.5	0.5	102-194	145	1.7	98.3
<i>koliensis</i> ....	20-71	43	1.6	98.4	51-119	75	99.5	0.5
<i>farauti</i> .....	17-55	28	33.4	66.6	71-180	109	22.6	77.4
Species	Hair 1 of Ab. VII Length							
	Range	Ave.	% under 111 mic.	% 111 mic. or over				
<i>punctulatus</i> .	115-204	152	0.6	99.4				
<i>koliensis</i> ....	64-112	85	99.5	0.5				
<i>farauti</i> .....	78-170	118	38.0	62.0				

\* Measurements in microns.

† Ab. stands for abdominal segment.

*Legs*: Light brown to black with yellowish-white markings. Front femora swollen in basal half, light at base, speckled and blotched with light along anterior and posterior surfaces; dorsal surface of tibiae with evenly spaced small light spots, ventral surface light, anterior surface dark, posterior surface speckled with light areas the majority of which connect with the light ventral area; first tarsal segment with light apex and variable number of light spots which expand laterally and ventrally, reducing the dark areas on ventral surface; second and third segments light at base and apex, central dark area may be greatly reduced ventrally; fourth segment light at base and usually apex; fifth segment light or dark; segments two to four may have light spots in central dark area; ventral surfaces of all segments usually much lighter than dorsal. Middle and hind femora and tibiae speckled. First segment of middle and hind tarsi similar to corresponding segment of front tarsi; second, third, and fourth segments with narrow apical light bands; fifth usually all dark; second and third segments frequently with one or more light spots in central dark area; ventral and posterior surfaces of segments one and two usually lighter.

*Abdomen*: Integument dark brown; vestiture of golden hairs, denser on posterior segments. Scales absent on tergites I to V and sternites I-VI, a few scales on tergites VI and VII and sternite VII; rather dense scaling on tergite and sternite VIII. Cerci with dark scales at base, light on apex.

*ADULT MALE*.—Labium with at least two or three, usually numerous, ventral light spots, frequently with large patches of light scales. Second morphological segment of palpus with light patch dorsally in basal half; articulation between second and third segments without scales,



TABLE 2.—Summary of diagnostic measurement data for pupae of *lungae* series

Species	Hair 1 of Ab. III Length				Hair 1 of Ab. IV Length			
	Range	Ave.	% under 17 mic.	% 17 mic. or over	Range	Ave.	% under 17 mic.	% 17 mic. or over
<i>lungae</i> ....	6-17	12	95.0	5.0	6-17	12	100.0	0.0
<i>solomonis</i> .	10-28	19	12.2	87.8	17-41	24	4.2	95.8
Species	Hair 1 of Ab. V Length							
	Range	Ave.	% under 25 mic.	% 25 mic. or over				
<i>lungae</i> ....	10-24	14	100.0	0.0				
<i>solomonis</i> .	27-75	58	0.0	100.0				

light-colored; third segment dark on basal fifth, light-scaled to apical fourth, remainder dark except light preapical area extending laterally to apex or apex light; segments four and five narrowly dark at or near base, dark area extending on outer surface usually to apex of segments, remainder light. Claws normal.

*Genitalia:* As figured by Ross and Roberts (1943).

TABLE 3.—Branches of diagnostic hairs of pupa

Species	Ab. I				Ab. II			
	Hair 6		Hair 9		Hair 5		Hair 10	
	Average	Range	Average	Range	Average	Range	Average	Range
<i>hollandi</i> .....	9	7-11	3	2-4	6	5-9	7	4-11
<i>solomonis</i> .....	7	5-9	2	1-4	3	2-5	6	5-8
<i>lungae</i> .....	6	5-7	1	1-2	3	1-4	7	5-10
<i>punctulatus</i> .....	5	3-8	1	1-1	1	1-2	18	7-38
<i>koliensis</i> .....	5	4-6	1	1-1	1	1-1	5	2-9
<i>farauti</i> .....	4	1-5	1	1-2	1	1-3	7	4-16
Species	Ab. III				Ab. IV			
	Hair 5		Hair 10		Hair 2		Hair 10	
	Average	Range	Average	Range	Average	Range	Average	Range
<i>hollandi</i> .....	8	5-10	10	7-14	3	2-4	8	6-10
<i>solomonis</i> .....	3	2-5	4	3-5	2	1-2	4	2-5
<i>lungae</i> .....	2	1-2	4	3-6	2	1-3	3	2-5
<i>punctulatus</i> .....	1	1-3	10	4-16	1	1-2	6	4-8
<i>koliensis</i> .....	1	1-2	10	7-13	1	1-2	7	5-9
<i>farauti</i> .....	1	1-2	8	5-11	1	1-1	6	3-8
Species	Ab. V				Ab. VI			
	Hair 10		Hair 2		Hair 6		Hair 10	
	Average	Range	Average	Range	Average	Range	Average	Range
<i>hollandi</i> .....	5	4-7	3	1-4	7	5-11	5	3-7
<i>solomonis</i> .....	3	2-4	2	2-3	3	2-4	2	2-4
<i>lungae</i> .....	3	2-4	2	1-3	4	3-5	2	1-4
<i>punctulatus</i> .....	3	2-4	2	1-3	4	3-5	1	1-3
<i>koliensis</i> .....	4	3-6	1	1-1	4	2-5	2	1-3
<i>farauti</i> .....	3	1-6	1	1-2	4	3-6	2	1-4
Species	Ab. VII				Ab. VIII			
	Hair 6		Hair 8		Hair 10		Hair 8	
	Average	Range	Average	Range	Average	Range	Average	Range
<i>hollandi</i> .....	7	4-8	6	3-10	5	4-6	4	3-7
<i>solomonis</i> .....	3	1-4	5	2-8	2	1-4	2	2-3
<i>lungae</i> .....	4	2-6	4	3-6	1	1-2	2	2-3
<i>punctulatus</i> .....	4	3-5	4	2-5	1	1-2	2	1-3
<i>koliensis</i> .....	4	3-5	3	2-4	1	1-2	3	2-4
<i>farauti</i> .....	4	3-6	3	2-4	1	1-2	2	1-3

PUPA.—Tables 1 and 3 contain the data for the more important hair counts and measurements of this species.

*Cephalothorax*: Trumpet pale, with only slight pigmentation in the central area between the apex and the basal notch, offering little contrast to the cephalothorax. Index of width to length of head shield 1:0.90 (range 0.81–1.0). External margin of palpal case usually angulate. Internal margin of median keel sharply outlined. Metanotum with a distinct, smooth median bar, bounded laterally by a crease which is incomplete posteriorly. Details of chaetotaxy not noticeably different from those of *solomonis*.

*Abdomen*: On segment II, hair 2 on or very near to a longitudinal line through hair 3; on segment IV, hair 5 external to hair 8; on segments VI and VII, hair 2 distinctly anterior to the posterior margin of the segment. Lateral spine (hair 1) of segment IV approximately equal to the length of that of segment III; lateral spine of V several times the length of that on III, and two-thirds to three-fourths the length of that on VI; lateral spines of V–VII (Fig. 26, H) rarely branched, frayed or with irregular outline, extremely elongate and slender. Similar to *solomonis* in the remaining details of structure and chaetotaxy.

*Paddle*: Midrib with external margin distinct, completely without granulations in over 50 per cent of the specimens examined, when granulations present seldom completely filling the area of the midrib but arranged in slender skeins along its length. Buttress well developed. Fringe of fine pale hairs extending from center of external margin to a short distance internal to hair 12, longest just before this hair. Paddle hair (hair 12) straight or curved, rather stout, darkly pigmented.

*Genitalia*: Apex of lobes in male shaped as shown in Fig. 17. Female genitalia as in Fig. 21.

LARVA.—Anterior clypeal hairs unbranched, the outer half or less the length of the inner. Posterior clypeals single, usually long enough to reach the tubercles of the anterior clypeals. Occipitals long, inner single or double; outer 1–3 (rarely 4)-branched. Tubercles of prothoracic hairs 1 and 2 small, unpigmented, separated from one another; stem of hair 1 slender, at most slightly enlarged basally. Long thoracic pleural hairs all single. Hair 1 of abdominal segment I with short, hair-like branches. Palmate hairs present on segments II–VII, all small, especially on segment II; filament of leaflet almost as long as the shaft. Hairs 2 and 6 of abdominal segments IV and V usually 3-branched.

*Taxonomic Discussion*.—*A. punctulatus*, *farauti*, *koliensis*, and *moluccensis* form a closely related complex of species in the group *Neomyzomyia*. The combination of the presence of scales on the disc of the scutum, the absence of scales from all but the terminal abdominal segments, the speckling of the legs, and the broad apical light bands on the last two palpal segments (3rd palpal segment variable) separate the *punctulatus* series from all other *Neomyzomyia* in the Oriental and Australasian regions. The larvae can usually be recognized from other *Neomyzomyia* with unbranched pleural hairs by the single or frayed widely spaced inner clypeals, by the single, frayed, or occasionally branched outer clypeals, which are never minute, and by the posterior clypeals which at most barely project beyond the margin of the head. *A. watsoni* and *hackeri* exhibit similar characters but can be separated by the position of the clypeal hairs, the bases of the inner, outer, and posterior clypeals on one side forming an equilateral, scalene or isosceles triangle.

The larva of *moluccensis* from New Guinea appears to be similar in structure to that of *farauti*, in that the tubercles of the prothoracic submedian hairs are fused and hair 1 of abdominal segment I is distinctly palmate with flattened leaflets. However, according to Swellengrebel and Rodenwaldt (1932) the adults of this species have the small light ventral patch of the labium which is characteristic of *koliensis*. *A. koliensis* and *moluccensis* larvae are easily separated from one another. Until comparison can be made with the type of *moluccensis* and with reared material from New Guinea, the exact relationship of *moluccensis* to *farauti* and *koliensis* cannot be determined.

It has been suggested that hybridization occurs between various members of the *punctulatus* series. Although no conclusive cross-breeding experiments have been made, the fact that the progeny of all species except of one female *koliensis* bred

true indicates that hybridization in the area in question does not normally occur in nature even though the species exist side by side. *A. punctulatus*, *farauti*, and *koliensis* can be separated from one another in the adult, pupal and larval stages. Therefore, we prefer to treat these forms as species, and not as subspecies of *punctulatus*.

*Distribution*.—GUADALCANAL. North coast: Tenamba, Umasami, Tassafaronga, Bonegi, Poha, Kokumbona, Matanikau, Lunga, Tenaru, Koli, Tetere; Southwest coast: West Cape. BOUGAINVILLE. Empress Augusta Bay (A. B. Gurney).

*Biology*.—On Guadalcanal, this species is restricted to the valleys of the larger streams and rivers, usually occurring in the vicinity of native villages away from the coast. Larvae have been collected most frequently exposed to sunlight in road ruts and other temporary pools such as depressions and footprints in native trails. The margins of streams and sloughs in exposed situations and pot holes in drying stream beds are also utilized occasionally, particularly during the dry season. The pools in which this species occurs may be entirely free of vegetation and flottage and frequently are very muddy, or may have marginal herbaceous vegetation and dense algal growth. *A. punctulatus* has a very decided preference for breeding in sunlight, but is also found in partial shade.

During periods of dry weather this species almost disappears as its chief breeding places are greatly reduced. At such times it resorts to breeding in streams. After an occasional heavy rain it appears in large numbers in temporary pools. During the rainy season *punctulatus* extends its range into the coastal plain near the mouths of the rivers and utilizes the same breeding places as *farauti*.

The larvae and pupae can usually be recognized in the field by the uniformly light opaque coloration of the body. They are extremely active, especially when crowded in small breeding places without vegetation and seem to spend a great deal of time under water in search of food.

Some information on the length of the aquatic cycle of *punctulatus* and *farauti* under natural conditions was obtained on Guadalcanal in early March 1944. With relatively low water temperature (70°–91° F.), high rainfall and unfavorable food supply (old oil film and euglenoid film), the larval stage was observed to last five days. Approximately one day was required for the completion of each of the first three instars, and two days for the fourth instar. As the eggs took slightly less than two days to hatch and the pupal stage lasted approximately 20 hours, the entire aquatic cycle from oviposition to emergence of the adults was accomplished in less than eight days. Under more favorable conditions it undoubtedly is reduced to less than a week. The possibility that eggs are laid in mud was indicated by the appearance of first instar larvae in isolated temporary pools less than a day after they had become filled with water. Puddles with larvae and pupae were observed to dry completely; when these were filled two days later fourth instar larvae and pupae appeared. It would appear from these observations that *punctulatus* and *farauti* are well adapted to breeding in temporary pools and may be able to survive at least short periods of dry weather in the egg and possibly also the larval and pupal stages. Further indications of the short aquatic cycle of *punctulatus* and its adaptation to breeding in temporary pools were obtained from observations on a colony of this species maintained for a period of three months during the dry season. Gravid females would not lay eggs in small water con-

ainers, but scattered them on the floor of the cages. Eggs were collected in large numbers on moist filter paper placed on the bottom of the cage. Hatching took place in less than two days after oviposition, all the first instar larvae appearing in less than one hour of each other. A strikingly uniform rate of development of the larvae was observed in this species and was different from all other local anophelines. At any one time in a batch of the same age, the larvae would be of the same instar and size, taking from six to seven days to pupation with adequate food supply.

The short time required for *punctulatus* and *farauti* to complete the aquatic cycle in nature is of considerable interest in relation to control operations directed against these species.

Although females of *punctulatus* feed readily in captivity, they do not appear to attack man often in nature on Guadalcanal. Less than one per cent of the total anophelines collected in routine night catches for a period of over a year have been *punctulatus*. Special efforts were made to collect adults in native villages and in the open in areas of high larval densities of this species on the northwest coast of Guadalcanal with the result that a single blooded female was obtained in a native hut, while *farauti* was collected at will in the open as well as in dwellings. On the northeast coast *punctulatus* has been collected in native camps and villages, but in smaller numbers than *koliensis* or *farauti*.

The diurnal resting places of the adults of this species are not known on Guadalcanal. They have been searched for on stream banks, in jungle areas near breeding places, and in the vicinity of native villages.

#### *Anopheles (Myzomyia) farauti* Laveran

1902. *Anopheles Farauti* Laveran, C. R. Soc. Biol. Paris 54: 908 (♀♀ only). Type locality: Faureville, Ile Vate, New Hebrides. Types: unknown.
1927. *Anopheles (Myzomyia) punctulatus* Doenitz. Buxton and Hopkins, Res. in Polynesia and Melanesia, p. 67.
1929. *Anopheles punctulatus* Doenitz. Paine and Edwards, Bull. Ent. Res. 20: 304.
1931. *Anopheles punctulatus* Doenitz. Senevet, C. R. 2e Congr. Intern. Paludisme, Institut Pasteur, Alger. 1: 109.
1944. *Anopheles punctulatus farauti* Laveran. Knight and Farner, Proc. Ent. Soc. Wash. 46: 132.
1944. *Anopheles farauti farauti* Laveran. Knight, Bohart and Bohart. Keys to the Mosquitoes of the Australasian Region. Nat. Res. Council, Wash., p. 11.

**ADULT FEMALE.**—In general as in *punctulatus*. Labium entirely dark-scaled except for narrow light ring at extreme apex; a few light scales rarely present on basal half. Third morphological palpal segment usually with narrow apical light ring separated from larger subapical light area by dark ring; these pale markings, especially the subapical spot, frequently reduced, sometimes subapical completely absent. Small dark sectoral spot usually present on costa between basal and median dark spots. Buccopharyngeal armature as in *punctulatus*.

**ADULT MALE.**—In general as in *punctulatus*. Labium entirely dark-scaled except for narrow light ring at extreme apex. Small dark sectoral spot usually present on costa between basal and median dark spots.

**Genitalia:** Similar to those of *punctulatus*.

**PUPA.**—The three terminal abdominal segments of this species were figured by Buxton and Hopkins (1927) from New Hebridean specimens. Senevet (1931) described and illustrated the dorsal and ventral chaetotaxy of an anopheline from Iles Salomon (Solomon Islands?) which he identified as *punctulatus*. However, from the incomplete figure given by Senevet, it appears that his specimens were *farauti*. Tables 1 and 3 contain the data for the more important hair counts and measurements made for this species. Similar to the pupa of *punctulatus* except for the following:

**Cephalothorax:** Trumpet orange-brown, contrasting noticeably to cephalothorax, pigmented area extending basad from the apex at least to the basal notch. Index of width to length of head shield 1:0.88 (range 0.65–1.11).



*Abdomen:* On segment IV, hair 5 internal to or in line with hair 8. Lateral spine (hair 1) of segment IV approximately twice the length of that of III; lateral spines of segments V–VI may occasionally have one or more minute lateral branches, or even be terminally frayed; lateral spine of segment VII (Figs. 26, D and E) elongate, stout, acutely tapered, usually branched or frayed; frequently those without obvious branching give the impression of possessing branches that are not separated from the shaft.

*Paddle:* Midrib usually evenly granulated.

*LARVA.*—Anterior clypeal hairs usually frayed; outer at least half the length of the inner; posterior clypeals long, extending beyond the anterior tubercles, usually single, sometimes 2-branched. Occipital hairs short; inner 1–2 (rarely 3)-branched, outer 1–4 (rarely 5–6)-branched. Tubercles of prothoracic hairs 1 and 2 long, pigmented, and almost always fused; stem of hair 1 swollen basally. Hair 1 of abdominal segment I a definite palmate tuft, with flattened, notched leaflets. Palmate hairs also present on segments II–VII; those on segment II pigmented and only slightly smaller than those on segment III. Leaflets with long filaments, sometimes almost the length of the shaft. Hairs 2 and 6 of abdominal segments IV and V usually double.

*Variation.*—On Guadalcanal in areas where there is little chance for human contact such as near abandoned native villages or campsites and beyond the edge of the controlled territory, the larvae of *farauti* are somewhat different from those reared in the laboratory in that the outer clypeals and outer occipitals exhibit heavier branching. A similar condition exists in more darkly pigmented larvae collected in the control area. Adults bred from larvae collected in such areas do not feed readily on humans. This seems to indicate the presence on Guadalcanal of a “wild” strain of *farauti*.

*Taxonomic Discussion.*—See under *A. punctulatus*.

*Distribution.*—NEW HEBRIDES: EFATE. Faureville (Laveran, 1902), Teuma and Havannah Harbor (Buxton and Hopkins, 1927), generally distributed along the coastal areas. ESPIRITU SANTO. Big Bay (Buxton and Hopkins, 1927), generally distributed along the southeastern and southern coastal area. AORE. MALO. TANNA. Whitesands (Buxton and Hopkins, 1927). MAI. (Buxton and Hopkins, 1927). MALEKULA. Tisman (Buxton and Hopkins, 1927), Port Sandwich. SOLOMON ISLANDS: GUADALCANAL. Generally distributed along the entire coast, has been collected on the north coast from Marovovo to Aola Bay, and at West Cape on the southwest coast. MALAITA (S. M. Lambert, J. R. Douglas). TULAGI. FLORIDA. SAVO. RUSSELL ISLANDS (W. G. Downs). NEW GEORGIA GROUP (W. G. Downs, J. G. Franclemont). BOUGAINVILLE. Empress Augusta Bay (A. B. Gurney). TREASURY ISLANDS (J. H. Paullus).

*Biology.*—The natural breeding places of this species consist primarily of river and stream margins with vegetation, springs, wells, seepage areas, taro gardens, ponds, lagoons, and swamps, all in open coastal areas. Even when these breeding areas are greatly reduced *farauti* does not invade such habitats as undisturbed dense canebrake marshes, or large open sunlit swamps in the jungle filled with floating and emergent vegetation. Normally, the breeding does not extend far from settled areas, usually not more than a mile, unless the jungle is cleared. However, breeding will often persist in areas once occupied, but later abandoned. Following heavy rains any of the very numerous temporary pools formed in the open coastal areas are utilized by this species. These may be natural or man-made water-holding depressions such as puddles, hog wallows, ruts, holes, hoofprints, bases of uprooted trees, borrow pits, poorly graded ditches and occasionally holes in coral above high tide mark. Frequent floods of the larger streams and rivers eliminate them as important breeding places during periods of high rainfall. When the species is denied breeding areas on the ground or when it becomes extremely abundant, it



resorts to breeding in artificial containers to a limited degree. At such times it has been collected in boats, tanks, oil drums, water collections in canvas, and even small tin cans.

The aquatic stages of *farauti* have a definite preference for sunlight, but are very frequently collected in large numbers under partial shade, rarely being found in very dense shade. This species tolerates a wide range of conditions, utilizing all types of water from highly turbid to clear spring water, from stagnant, foul or brackish to fresh rain water. It is not found in water collections in plants, such as leaf or frond axils, cocoanut shells, cacao pods, or tree holes, nor does it occur in water with high organic content, such as is found in jungle swamps. In almost all cases, breeding in extensive water areas is associated with flotage and emergent or surface vegetation. However, in small confined places such as pools, puddles, and road ruts, larvae will commonly be found on the open surface.

*A. farauti* is frequently associated with *punctulatus* where the latter is present in temporary pools during the rainy season. In the dry season it is found together with *B. hollandi* and *A. lungae* along the more exposed stream margins. The culicines most frequently associated with *farauti* are *Culex annulirostris* Skuse, *C. basincinctus* Edw. (New Hebrides), *C. squamosus* (Taylor), (Solomons), *C. jepsoni* Theo., *C. pullus* Theo. (Solomons), and during the rainy season various species of flood-water *Aedes*.

Larvae collected in the field frequently have a dark pigmentation mottled with light areas, but they may be just as light as those of *punctulatus* or greenish depending on the type of breeding place. Usually the antennae are darkly pigmented.

The aquatic cycle appears to be very short. It has been discussed under *punctulatus*.

Adult females enter any type of shelter in search of human blood, and readily bite out of doors. Feeding takes place generally from dusk to dawn. However, a number of daytime biting records have been obtained. In most cases, these attacks were in covered or shaded areas, but a few were made directly in bright sunlight. The flight is relatively quiet and the females are shy and wary in their approach, easily frightened away by movements, but persistent in returning to attack. In houses or tents where lights were present, they will feed most readily in dark corners and on shaded portions of the body. Their bite is noticeably painless to many individuals. Throughout the area covered by this report, except for isolated areas in the Koli district on Guadalcanal, *farauti* is the principal anopheline attacking man. On the northwest coast of Guadalcanal, 98 per cent of anophelines in routine night catches conducted for a period of over a year were this species.

The diurnal resting places for males and females apparently consist of nearly any cool, moist and shaded spot. Adults of *farauti* have been collected on Guadalcanal resting on buttressed tree trunks and under logs in swampy jungle areas in association with *lungae* and *solomonis*, but never in large numbers. In the New Hebrides they have been found among roots of banyan trees, weeds and grass, in open barrels, tin cans and wooden boxes, on shaded, moist earthen walls of pits and holes, and in moist situations beneath large felled logs. Resting blooded females may be collected at will in occupied houses and native huts, the sites being generally dark and somewhat secluded, such as in the folds of clothing and mosquito nets, on the undersurface of furniture, rafters and hanging objects and upon walls and low ceilings. They are commonly found from the ground surface to a height

of seven feet. On Guadalcanal they have been collected in native huts and tents under extremely high temperature in the middle of the day.

No definite information is available on flight range. Our records show several instances of adults having been captured at least one mile from the nearest known breeding area. On Espiritu Santo a blooded female was found by R. H. Daggy on a boat 600 yards off shore in the channel. Adults are commonly taken up to 400 yards from breeding areas.

Although no comprehensive data are available on the longevity of *farauti*, several females were kept alive in a small laboratory cage for approximately 35 days. The males, which were introduced at the same time, survived less than two weeks. Both insectary and cage mating have been obtained with this species. Although on Espiritu Santo and Efate colonies were readily established, such attempts on Guadalcanal met with great difficulty. This may be due in part to that fact that larvae were collected along the margin of the control area in places where human blood was not available. Under the section on variation above, the differences in the morphology of larvae found in such areas are discussed and the possibility of the existence of a "wild" strain of *farauti* is suggested.

*Anopheles (Myzomyia) koliensis* Owen

1945. *Anopheles punctulatus koliensis* Owen, 1945. Type locality: Koli area, Guadalcanal, Types: U. S. National Museum.

ADULT FEMALE.—In general as in *punctulatus*. Apical third of labium with ventral area of pale scales; usually a well-defined ventral patch, which may extend dorsally in form of pale band at its anterior extremity; rarely pale markings reduced to a few scales ventrally; very rarely apical third dark-scaled as in *farauti* or extensively pale-scaled as in *punctulatus*; dorsal pale area when present not as extensive as in *punctulatus*; a few pale scales in basal half of labium may be present. Usually no small dark sectoral spot on costa between basal and median dark spots. Dark coloration of legs usually more extensive than in *punctulatus*, extending to ventral surface of tarsal segments. Buccopharyngeal armature as in *punctulatus*.

ADULT MALE.—In general as in *punctulatus*. Labium usually entirely dark-scaled except for a narrow apical light ring at extreme apex. Wings and legs as in female *koliensis*.

*Genitalia*: similar to *punctulatus*.

PUPA.—The pupa of this species is quite similar to that of *farauti*. Tables 1 and 3 give the data for the hair counts and measurements that have diagnostic value.

*Cephalothorax*: Index of width to length of head shield 1:0.88 (range 0.76–1.0).

*Abdomen*: Hair 5 of segment IV is frequently (35 per cent) external to hair 8, whereas in *farauti* it is in line or internal to hair 8 (91 per cent). Lateral spine of segment 7 (Fig. 26, F) stout, acutely tapered from base, only rarely branched, but when so of a distinctive type (Fig. 26, G).

*Paddle*: Midrib incompletely granulated, or occasionally granules entirely missing.

*Genitalia*: Apices of lobes in male distinctively shaped (Fig. 18).

LARVA.—Anterior clypeal hairs either simple or slightly frayed; outer half or more the length of the inner; posterior clypeals single and very short, not extending to the tubercles of the anterior hairs. Occipital hairs short; inner 2–4-branched; outer usually 5–9-, occasionally 3–4-branched. Tubercles of prothoracic hairs 1 and 2 usually separated; stem of hair 1 swollen. All long hairs of thoracic pleural groups single. Hair 1 of abdominal segment I with long, narrow, hair-like branches. Palmate hairs present on segments II–VII, those on segment II only slightly smaller than those on segment III. Filament of leaflets almost as long as the shaft. Hairs 2 and 6 of abdominal segments IV and V usually 2, sometimes 3-branched.

*Taxonomic Discussion*.—See under *A. punctulatus*.

*Distribution*.—GUADALCANAL. North coast from Malimbu River east to Aola Bay (W. B. Owen); absent from the northwest coast.

*Biology*.—Owen collected the larvae of this species in water exposed to sunlight from temporary pools in grasslands and along the edge of the jungle in association with *farauti* and once in association with both *farauti* and *punctulatus*. They were scarce in comparison with the other anophelines collected. The adults are

strongly anthropophilic. Where blooded females have been collected in the daytime resting in native huts and tents they have formed 90 per cent of the anopheline population. During the dry season they become active about 9:00 PM, the greatest activity occurring after midnight and continuing until daylight (W. B. Owen).

## REFERENCES CITED

- BELKIN, J. N. AND SCHLOSSER, R. J. 1944 A new species of *Anopheles* from the Solomon Islands. Jour. Wash. Acad. Sci. 34: 268-273.
- BUXTON, P. A. AND HOPKINS, G. H. E. 1927 Researches in Polynesia and Melanesia. Parts I-IV. Lond. School Hyg. and Trop. Med. Memoir Series No. 1. 260 pp. 12 pls. 43 figs.
- LAVERAN, N. A. 1902 Sur les culicides des Nouvelles-Hébrides. C. R. Soc. Biol. Paris 54: 908-910.
- OWEN, W. B. 1945 A new anopheline from the Solomon Islands with notes on its biology. J. Parasitol. 31: 236-240.
- ROSS, E. S. AND ROBERTS, H. R. 1943 Mosquito atlas. Part II. Eighteen Old World anophelines important to malaria. 44 pp. 36 pls. Amer. Ent. Soc., Philadelphia.
- SENEVET, G. 1931 Contribution à l'étude de nymphes de culicides. Description de celles de certains anophélins et plus spécialement de espèces européennes et Méditerranéennes. C. R. 2e Congr. Intern. Paludisme; Instit. Pasteur, Alger. 1: 109.
- SWELLENGREBEL, N. H. AND RODENWALDT, E. 1932 Die Anophelen von Niederlaendisch-Ostindien (3rd Ed.). 242 pp. 24 pls., 24 maps, 42 figs. Jena.
- TAYLOR, F. H. 1934 The Diptera of the Territory of New Guinea. I. Proc. Linn. Soc. N. S. Wales 59: 229-236.
- VAN HELL, J. C. 1938 Een vergelijkende studie van de pleuraharen van de Nederlandsch-Indische anopheleslarven. Meded. Dienst. Volk. Ned.-Ind. 27: 476-492.

## EXPLANATION OF PLATES

## PLATE I—Egg and Adult

- FIG. 1. *A. solomonis*—Wing.
- FIG. 2. *A. solomonis*—Head, proboscis and palpus.
- FIG. 3. *B. hollandi*—Male genitalia.
- FIG. 4. *B. hollandi*—Lateral aspect of claspette.
- FIG. 5. *A. lungae*—Buccopharyngeal armature.
- FIG. 6. *A. solomonis*—Buccopharyngeal armature.
- FIG. 7. *B. hollandi*—Egg.

## PLATE II—Larva

- FIG. 8. *B. hollandi*—Head of larva.
- FIG. 9. *B. hollandi*—Prothoracic submedian group.
- FIG. 10. *B. hollandi*—Ventral aspect of left prothoracic pleural group.
- FIG. 11. *B. hollandi*—Ventral aspect of left mesothoracic pleural group.
- FIG. 12. *B. hollandi*—Ventral aspect of left metathoracic pleural group.
- FIG. 13. *A. solomonis*—Ventral aspect of left prothoracic pleural group.
- FIG. 14. *A. solomonis*—Ventral aspect of left metathoracic pleural group.
- FIG. 15. *A. lungae*—Ventral aspect of left prothoracic pleural group.

## PLATE III—Pupa

- FIG. 16. *B. hollandi*—Dorsal and ventral aspect of metanotum and abdomen.
- FIG. 17. *A. solomonis*—Male genitalia.
- FIG. 18. *A. koliensis*—Male genitalia.
- FIG. 19. *B. hollandi*—Paddle.
- FIG. 20. *A. solomonis*—Paddle.
- FIG. 21. *A. farauti*—Female genitalia.
- FIG. 22. *B. hollandi*—Male genitalia.
- FIG. 23. *B. hollandi*—Trumpet.
- FIG. 24. *B. hollandi*—Female genitalia.
- FIG. 25. *B. hollandi*—Lateral spine (hair 1) of abdominal segment VII.
- FIG. 26, A. *A. solomonis*—Lateral spine of abdominal segment VII.
- FIG. 26, B and C. *A. lungae*—Lateral spine of abdominal segment VII.
- FIG. 26, D and E. *A. farauti*—Lateral spine of abdominal segment VII.
- FIG. 26, F and G. *A. koliensis*—Lateral spine of abdominal segment VII.
- FIG. 26, H. *A. punctulatus*—Lateral spine of abdominal segment VII.
- FIG. 27. *B. hollandi*—Anterior portion of cephalothorax.

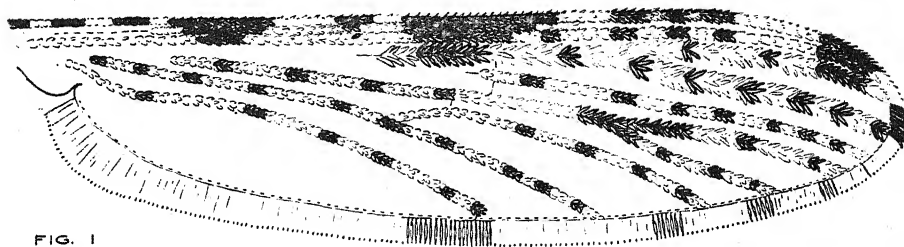


FIG. 1

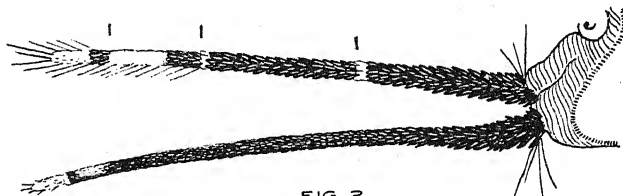


FIG. 2

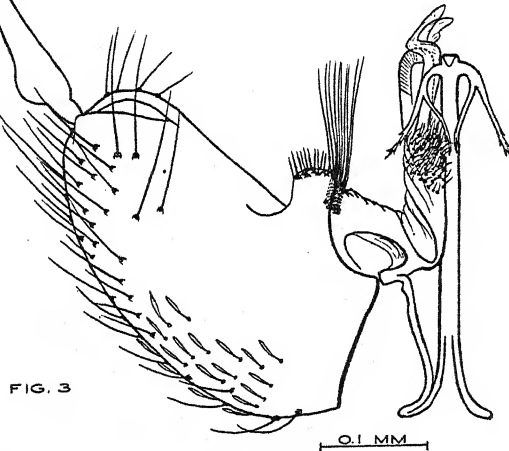


FIG. 3



FIG. 4



FIG. 5



FIG. 6

0.035 MM

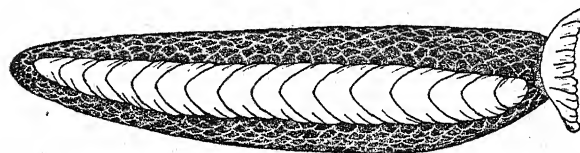


FIG. 7

0.1 MM







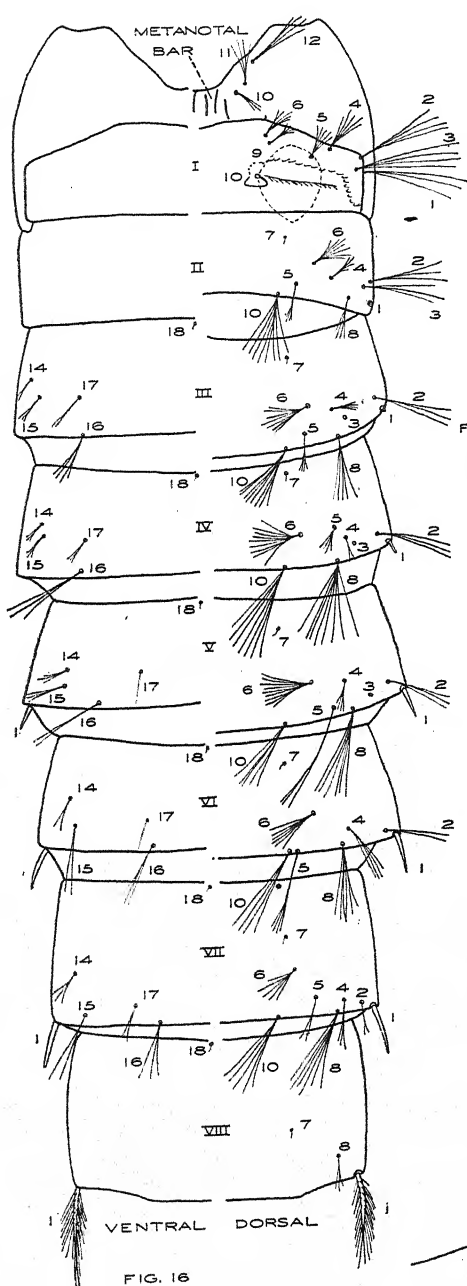


FIG. 16

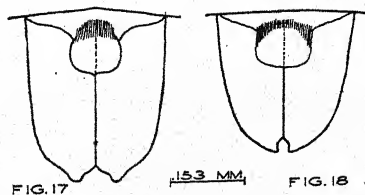


FIG. 17

FIG. 18

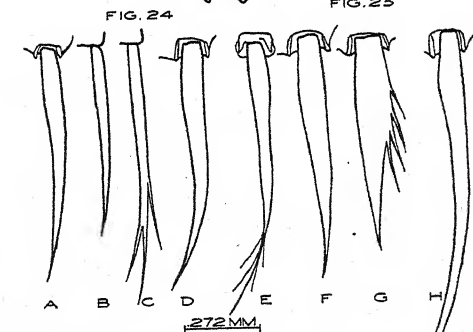
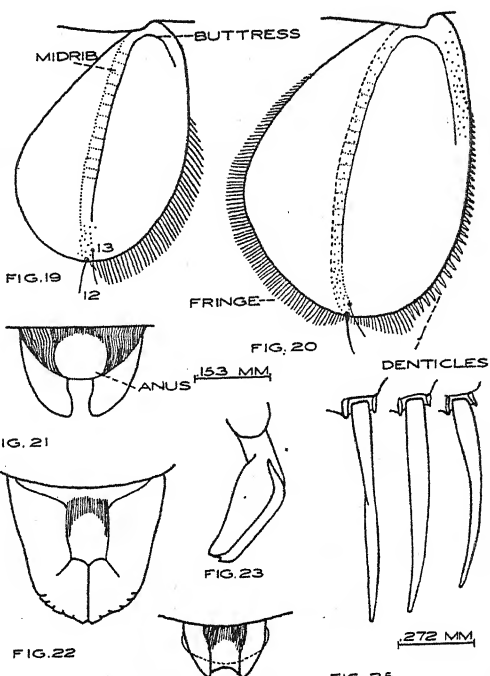


FIG. 26

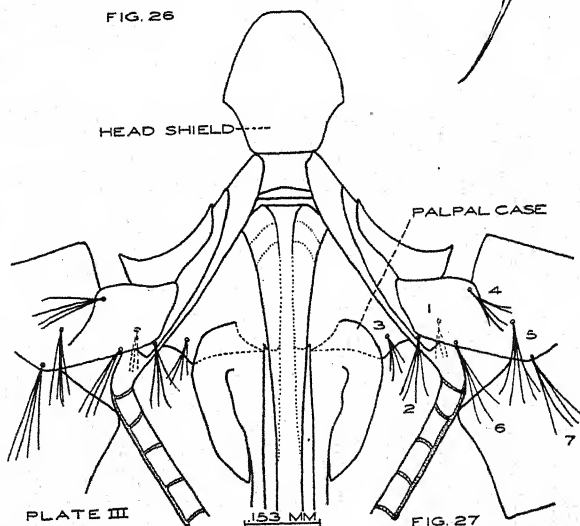


PLATE III

FIG. 27

# EXPERIMENTS TO DETERMINE WHETHER INFECTIVE LARVAE OF *WUCHERERIA BANCROFTI* CAN MIGRATE FROM THE ABDOMEN OF THE MOSQUITO INTERMEDIATE HOST

WALTER L. NEWTON AND IVAN PRATT

Zoology Laboratory, National Institute of Health, U. S. Public Health Service

In the course of dissecting mosquitoes experimentally infected with *Wuchereria bancrofti*, the authors found many specimens containing infective larvae in sites other than the head and proboscis, particularly in the abdominal cavity. It was of practical interest to find out whether or not infective larvae occurring in the abdominal cavity are capable of migrating forward to the proboscis. If free migration within the mosquito could be demonstrated, it could be assumed that infective larvae occurring anywhere within the mosquito could reach the proboscis and thereby be involved in transmission.

In the event that infective larvae occurring within the abdominal cavity do not migrate forward, it should be possible to demonstrate one or more of the following phenomena: That there is some anatomical barrier to the forward progress of the larvae once they have reached the abdomen, that there is some physiological reaction on the part of the mosquito which engulfs or kills the larvae, or that migration on the part of the larvae is indifferent; i.e., there is a general migration away from the thorax, but once the larvae have escaped this area there is no impulse to migrate elsewhere.

No anatomical barrier has been observed. To the contrary, the open-type circulatory system of the mosquito would seem to provide an excellent passage for forward migration of the larvae from the abdomen. There has been no evidence of encapsulation or death of the larvae within the abdomen of mosquitoes that permit development to the infective stage. Finally, mosquitoes dissected shortly after the larvae have become infective contained most of the larvae in the abdominal cavity; whereas, specimens kept several days longer contained fewer larvae in the abdomen with a greater proportion of them in the thorax, head, and proboscis. This would indicate that larval migration is not aimless and that there is a tendency for larvae in the abdominal cavity to migrate forward toward the head.

In view of the above, it was the opinion of the authors that infective larvae of *Wuchereria bancrofti* were both capable of and inclined to migrate out of the abdominal cavity toward the head and proboscis.

There has been little, if any, experimental work to prove or disprove the theories regarding the migration of infective *Wuchereria bancrofti* larvae in the mosquito host. Yamada (1927) suggested that the infective larvae migrated from the thorax to the abdomen and then to the head. Highby (1943), while working with *Dipet-*alonema arbuta**, actually observed the infective larvae migrating freely from one part of the body to another and from the proboscis back to the abdomen.

To provide data on this point, an experiment was set up to determine whether the larvae are able to make their way out of the abdomen. The technique consisted of transferring infective larvae to the abdomen of a non-infected mosquito. If the

larvae are capable of migrating and are prone to migrate, it should be possible to recover them in other parts of the body on subsequent dissection of the mosquito.

The operation was a delicate one and resulted in the injury or death of some of the transposed larvae. Small numbers of larvae were used in each test in order to minimize the chances of injuring the specimens by overcrowding in the pipette. After the removal of legs and wings, a short, longitudinal incision was made along the lateral abdominal wall of the recipient mosquito. By means of a capillary pipette, as much of the fluid as possible was removed from the cavity. A drop of saline containing 1 to 7 infective larvae was placed over the wound. As the drop evaporated and grew smaller, the larvae eventually worked their way down into the

TABLE 1.—Results of dissection of mosquitoes into which infective larvae of *Wuchereria bancrofti* were implanted

Experiment number	Recipient species	Number larvae implanted	Time elapsed before dissection (minutes)	Location of larvae recovered			
				Pro-boscis	Head	Thorax	Abdomen
1	<i>Psorophora confinnis</i>	4	20	1*	0	1	2**
2	<i>Culex tarsalis</i>	3	30	0	1	1	1
3	<i>Aedes aegypti</i>	2	50	0	0	1	1***
4	<i>Culex quinquefasciatus</i>	2	35	0	1	0	1
5	"	4	20	0	0	0	3
6	"	4	95	0	0	0	1**
7	"	4	40	0	3	0	1
8	<i>Anopheles quadrimaculatus</i>	3	55	0	0	1	3***
9	"	2	60	0	0	2	1
10	"	3	60	1	0	1	0
11	"	2	60	0	0	1	1**
12	"	3	60	0	0	2	1
13	"	3	90	0	0	2	1
14	"	3	60	0	0	3	0
15	"	2	60	0	0	1	1
16	"	2	90	0	0	0	2
17	"	4	60	2	0	1	1***
18	"	4	55	2*	0	2	0
19	"	2	90	0	0	0	2
20	"	2	75	0	0	2	0
21	"	4	70	0	0	1	3
22	"	7	60	0	0	5	1**
23	"	3	105	0	0	1	2
24	"	1	100	0	0	0	1
25	"	2	80	0	1	1	0
26	"	3	40	0	0	2	1
27	"	2	30	0	0	1	1

\* —One of these larvae was seen in the proboscis within 5 minutes.

\*\* —These were found to be injured.

\*\*\* —These were found to be dead.

abdomen. When the larvae were slow in entering the abdomen, water had to be added to the drop to prevent the salt solution from becoming too concentrated. Within a few minutes the incision closed over, sealing the larvae inside where they usually could be seen moving about. After a time lapse, usually an hour, the mosquito was separated into abdomen, thorax, head, and proboscis. These parts were then examined for the presence of larvae.

Of a total of 27 mosquitoes in each of which at least one live larva capable of migration was recovered upon dissection, 22 contained larvae that had migrated from the abdomen (See Table 1). Of the total of 68 active larvae recovered from all the mosquitoes dissected, 32 had migrated as far as the thorax and an additional 12 as far as the head or proboscis. Twenty-four active larvae were found in the abdomen and apparently had not migrated from that site.

It would appear that the amount of time required for the migration of the larvae to any particular part of the body was irregular. In one instance by means of a strong light a larva was seen in the proboscis 5 minutes after its introduction into the abdominal cavity. In another specimen larvae were found only as far as the thorax after 105 minutes.

#### SUMMARY AND CONCLUSIONS

Inoculations of infective larvae of *Wuchereria bancrofti* were made into the abdomen of previously uninfected mosquitoes to determine whether such larvae could migrate from this site to other parts of the body, particularly to the proboscis. The dissection of 27 mosquitoes so inoculated indicated that in 22 specimens the larvae had migrated from the site of injection. Of the total of 68 live larvae recovered, 32 had migrated as far as the thorax and an additional 12 as far as the head or proboscis.

The results indicate that the infective larvae of this species are capable of moving freely from the abdomen to other parts of the body of the mosquito intermediate host and that there is a definite tendency for such migrations to take place. Therefore infective larvae occurring anywhere in the body of the mosquito are potentially capable of transmitting infection to man.

#### REFERENCES

- HIGHBY, PAUL R. 1943 Mosquito vectors and larval development of *Dipetalonema arbuta* Highby (Nematoda) from the porcupine, *Erethizon dorsatum*. J. Parasitol. 29: 243-252.
- YAMADA, S. 1927 An experimental study on twenty-four species of Japanese mosquitoes regarding their susceptibility for *Filaria bancrofti* Cobbold. Sc. Rep. Gov. Inst. Inf. Dis., Tokyo Imp. Univ. 6: 559-622.

# THE MIRACIDIUM OF *PROTEROMETRA MACROSTOMA* (FAUST) HORSFALL 1933<sup>1</sup>

KATHLEEN L. HUSSEY

School of Public Health, Columbia University

In connection with another study on *Proterometra macrostoma* (Faust) Horsfall 1933, eggs with active miracidia were obtained. In trying to determine the number of flame cells of the unhatched miracidium, some were observed to hatch on the slide. By alternating treatment with salt and tap water and thus changing the osmotic pressure and slight coverglass pressure, several miracidia were obtained, which moved about actively.

## DESCRIPTION

The miracidium of *Proterometra macrostoma* is relatively large and pear-shaped, wider at the anterior end (Fig. 1). It is non-ciliated and has prominent bristle plates. At the anterior end these are arranged in five large patches of large bristles, (Figs. 2, 4) each with an associated band of smaller bristles (Figs. 2, 5) arranged in a somewhat spiral fashion. At the posterior end and extending nearly to the anterior patches are four longer bands of smaller bristles (Fig. 2), also arranged in a spiral fashion. A preparation impregnated with silver nitrate to show the boundaries of the dermal plates confirmed this observation, as there were five well-defined anterior plates and four posterior ones (Fig. 3), all rather widely separated from each other. Unfortunately only one miracidium was obtained in this preparation. The break and spur on one of the posterior plates may be (and probably is) an abnormality, but it was impossible to check. In the unhatched miracidium both Dickerman (1934) and Horsfall (1933, 1934) described only four anterior bristle patches. In the type of preparation studied, one bristle patch might have been overlooked.

The anterior central portion of the miracidium contains the so-called "primitive gut" showing four nuclei arranged in a linear series; laterally and posteriorly the miracidium is filled with germinal tissue; a single pair of flame cells is present (Fig. 1).

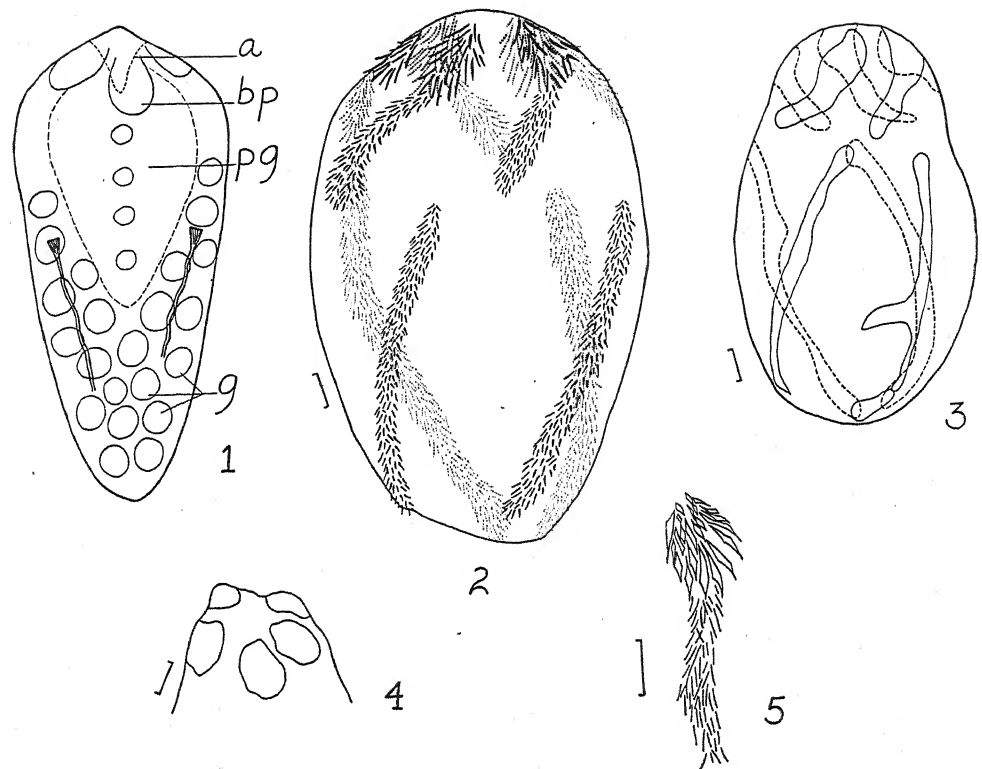
## ACTIVITY

Just before hatching, the miracidium becomes very active within the egg shell, moving around and rubbing against the inside of the shell. This activity is especially noticeable at the anterior end, with an alternate extension and withdrawal of the anterior proboscis-like structure and the anterior bristle patches. When the proboscis-like structure is withdrawn to its full extent (a, Fig. 1) the anterior large bristles point forward. The miracidium seems to swell and fill the shell more completely, pushes or stretches as if to force off the operculum, then finally pushes it off and creeps or flows out. When entirely free from the shell the miracidium moves about by a creeping movement very similar to that of an earthworm, elongating and contracting, the anterior end with its bristles acting as an exploring organ and possibly, in its contact with the substratum, aiding in locomotion. Since the

Received for publication, May 18, 1945.

<sup>1</sup> Contribution from the Department of Zoology, University of Michigan.





## EXPLANATION OF PLATE

Figures 2-5 drawn with the aid of the camera lucida. Scale—0.01 mm.

Figures 2, 4, 5 drawn from preparations stained with aceto-carmin and mounted in glycerine.

All figures of the miracidium of *Proterometra macrostoma*.

FIG. 1. Free-hand sketch of living miracidium. a—position of proboscis-like structure when withdrawn; bp—anterior large bristle plate; g—germinal tissue; pg—"primitive gut."

FIG. 2. Miracidium showing bristle plates.

FIG. 3. Outline showing dermal plates. Silver nitrate impregnation.

FIG. 4. Anterior end of miracidium, slightly oblique, showing relation of 5 anterior patches of large bristles.

FIG. 5. Detail of anterior patch of large bristles and its associated patch of small ones. Drawn bristle for bristle.

anterior bristles point backwards, this alternate extension and retraction of the anterior end is probably the method of penetration of the snail tissues.

## DISCUSSION

In its lack of cilia and presence of bristle plates this miracidium is similar to those of *Azygia lucii* (Looss, 1894), *Otodistomum cestoides* (Manter, 1926) and *Ptychogonimus megastomus* (Willemoes-Suhm, 1871), also of the family AZYGIIDAE. *Proterometra macrostoma* makes the fourth genus of the AZYGIIDAE in which the miracidium is non-ciliated and has bristle plates. A similar non-ciliated, bristled miracidium has also been reported for three species of the genus *Halipegus* (family HEMIURIDAE), *H. ovocaudatus* (Creutzburg, 1890), *H. eccentricus* (Thomas, 1939) and *H. amherstensis* (Rankin, 1944).

## REFERENCES

- \*CREUTZBURG, N. 1890 Untersuchungen über den Bau und die Entwicklung von *Distomum ovocaudatum* Vulpian. Inaug.—Dissert. Leipzig. 32 pp.
- DICKERMAN, E. E. 1934 Studies on the trematode family Azygiidae. I. The morphology and life cycle of *Proterometra macrostoma* Horsfall. Tr. Am. Micr. Soc. 53(1): 8-21.
- HORSFALL, M. W. 1933 Development of *Cercaria macrostoma* Faust into *Proterometra* (nov. gen.) *macrostoma*. Science 78(2017): 175-176.
- 1934 Studies on the life history and morphology of the cystocercous cercariae. Tr. Am. Micr. Soc. 53(4): 311-347.
- LOOSS, A. 1894 Die Distomen unserer Fische und Frösche. Bibliotheca Zool. 16: 296 pp.
- MANTER, H. W. 1926 Some North American fish trematodes. Ill. Biol. Monogr. 10(2): 7-138.
- RANKIN, J. S., JR. 1944 A review of the trematode genus *Halipegus* Looss, 1899, with an account of the life history of *H. amherstensis* n. sp. Tr. Am. Micr. Soc. 63(2): 149-164.
- THOMAS, L. J. 1939 Life cycle of a fluke, *Halipegus eccentricus* n. sp., found in the ears of frogs. J. Parasitol. 25(3): 207-221.
- WILLEMOES-SUHM, R. V. 1871 Ueber einige Trematoden und Nemathelminthen. Z. wissensch. Zool. 21: 175-203.

---

\* Original not seen.

# STUDIES ON FIVE NEW SPECIES OF XIPHIDIOCERCARIAE OF THE VIRGULA TYPE\*

PHILIP G. SEITNER

Department of Biology, Purdue University

## INTRODUCTION

Although larval trematodes have been investigated to a considerable extent in the United States, knowledge of certain types has been limited almost entirely to species reported from other countries. One such type is a particular kind of xiphidiocercariae, the oral sucker of which possesses not only a stylet but also a peculiar structure, the so-called "Virgula organ." In his scheme of classifying cercariae, Lühe (1909) placed larvae of this type in a separate group, the Cercariae Virgulae, named for *Cercaria virgula* Filippi, one of the first of its kind to be described. *Cercaria geddesi* Ameel, 1939, appears to be the only larva of the Virgula type described in the United States, although a number of species have been reported from other regions.

Cable (1939) reported the occurrence of an unidentified Virgula cercaria in the prosobranch snail, *Goniobasis depygis* (Say), collected from McCormicks Creek, Indiana. Over a period of several years, these snails have been collected in large numbers to provide various types of larval trematodes for instructional purposes. It has been found that the Virgula species occurs in snails collected at all times of the year and causes a higher incidence of infection than does any other of the several species that may be present. Because of the apparent rarity of Virgula cercariae in the United States and the scarcity of information concerning their life cycles, the abundance and availability of the species from McCormicks Creek prompted an investigation of its morphology and life history.

Later, collections of prosobranch snails from other sources within a limited radius of Lafayette, Indiana, revealed the presence of four other distinctly different species of the Virgula type, thus providing material for a comparative study of five new species.

## MATERIALS AND METHODS

The snails were collected from the various streams and placed in finger bowls. Those infected were isolated and kept alive as long as possible to provide cercariae for study. It was desirable to study larvae soon after emergence, since ageing ones showed numerous opacities and vacuolations that often obscured parts and hindered observation. Measurements and observations are from living specimens under light cover-glass pressure and either unstained or stained supravitaly with neutral red. Approximate maximum measurements are those of the larvae fully extended, minimum measurements those of the most contracted specimens. All measurements are in millimeters and incidences of infection are based on emergence of cercariae from isolated snails.

Received for publication, May 21, 1945.

\*The writer wishes to express his sincere thanks to Professor R. M. Cable for his guidance and encouragement throughout the present study, and to acknowledge the assistance of Miss Edna Banta in collecting snails, Dr. Henry van der Schalie who identified part of the molluscan material, and Miss Lois Kraus who provided infected snails found in connection with another study.

Prospective secondary intermediate hosts, chiefly May-fly naiads, were placed in dishes with infected snails for several days and examined at intervals for metacercariae, many of which could be detected in the living insect with a low-power binocular microscope. Infections were finally checked by dissecting the insects and removing metacercariae for study.

In the case of one species which readily penetrated and encysted in ephemeropterid naiads, metacercariae were permitted to develop until they were considered infective for the vertebrate host as determined by the appearance of the digestive ceca, clearing of the body and cessation of further development. Naiads containing these metacercariae were then fed forcibly to frogs, using a syringe-like glass tube and plunger. The frogs were maintained on cockroaches until killed later and examined for young worms.

#### OBSERVATIONS

Description of *Cercaria nyxetica* sp. nov. (Figs. 3-7)

*Snail host:* *Goniobasis depygis* (Say).

*Locality:* McCormicks Creek, Indiana.

*Incidence:* 5-50%, depending on the time of year.

*Specific diagnosis:* Distome xiphidiocercaria with characters of the *Virgula* group. Measurements of living cercariae are as follows: maximum length of body (extended) 0.2; minimum (contracted) 0.08; body averages 0.11 in length and 0.07 in width in slightly flattened specimens, neither greatly extended nor contracted. Tail 0.03 to 0.15 long depending on state of contraction, the moderately contracted tail measuring 0.07 long and 0.017 wide near the base. Oral sucker 0.04 in diameter; stylet 0.018-0.020 long and 0.006 wide at the base from dorsal (or ventral) aspect; *Virgula* organ 0.025 long and 0.04 wide at its anterior end. Diameter of pharynx 0.01, ventral sucker 0.02. Entire body spinose and provided with delicate setae set in small papillae which are more numerous anteriorly; tail with fine spines, those on the tip being slightly longer; contracted tail with deep marginal folds. Oral opening directed ventrad, oral cavity reduced by the large *Virgula* organ which is lighter in appearance than the sucker proper. Pharynx well developed; remainder of digestive system not apparent. Ventral sucker slightly posterior of middle of body. Genital primordium a C-shaped mass dorsal and slightly posterior to the ventral sucker. Three pairs of cephalic gland cells in posterior body half, with individual ducts opening near tip of stylet. Excretory vesicle U-shaped, wall lobate; tips of the cornua receive the main excretory tubules which extend forward, bifurcating slightly behind the level of the ventral sucker; complete flame cell pattern not determined. Develop in small, oval-round sporocysts, up to 0.3 in diameter.

The *Virgula* organ, which is the distinguishing feature of the group, may be discussed here, since its fate was traced farther in this species than in the others studied. In all of the species described in this paper, the organ appears to be a bilobed structure essentially in the form of a U with the two tapering cornua extending anteriorly, finally bending ventrally and converging to form a papilla. In larvae observed under light cover-glass pressure, this papilla can be seen to protrude into the oral opening. At its tip is a pore through which the secretion of the organ is expelled in droplets. Since the *Virgula* organ occupies the innermost part of the oral sucker, it has the appearance of a structure simply crowded into the oral cavity and pressed against the wall of the sucker without being actually embedded in it. In fact, it is difficult to say whether the inner membrane of the sucker does enclose the organ; if not, then it adheres very closely to the muscular portion of the sucker.

The *Virgula* organ is filled with a homogeneous substance which is oily in appearance and stains an orange or salmon color with neutral red. After discharge from the organ, this substance becomes granular, apparently due to its emulsification with water.

The function of the Virgula organ is obscure. Sewell (1922) suggested that it might serve as a support for the oral sucker during penetration of the second intermediate host. It seems, however, that this function would be a minor one in species which have diminutive Virgula organs. Furthermore, the discharge of the organ's contents as described above indicates a glandular function. This does not seem to be essential to penetration of the intermediate host, however, since the organ persists beyond penetration and even encystment and can be seen in young metacercariae.

The stylet (Fig. 4) is embedded in the sucker with the tip exposed and directed antero-ventrad. At about one-fourth its length from the tip, it bears a pair of dorsolateral, knob-like prominences or shoulders.

The anteriormost pair of cephalic glands is on a level with the ventral sucker. It and the pair immediately following are similar in appearance, with finely granular contents and ducts which open near the tip of the stylet. The posterior pair of cephalic glands is coarsely granular and stains darker with neutral red than do the others. Also, the ducts of the posterior pair open distinctly nearer the mouth.

The cercaria is very active. It swims with the body flexed ventrally and tail lashing rapidly. When not swimming, it creeps over the substratum in an inch-worm manner, using its suckers alternately.

The sporocysts (Fig. 7) occur in large numbers in the digestive gland of the snail. They are small, oval-round and colorless, and often contain about a dozen cercariae.

There is considerable seasonal variation in the incidence of infection. The greatest number of snails are infected during the late summer and autumn when the incidence was found to be close to fifty per cent. In spring and early summer, however, only five to ten per cent of the snails harbored the parasite.

#### *Notes on the Life History of Cercaria nyxetica*

*Cercaria nyxetica* has been found to penetrate and encyst in aquatic insect naiads of the orders Odonata and Ephemeroptera. The exact path of entry into these second intermediate hosts has not been determined. Metacercariae are widely distributed in the infected naiad, occurring throughout the abdomen, thorax and frequently in the femoral muscles of the legs. Encystment usually does not occur immediately and may be delayed as long as two days during which the larvae may be seen moving actively in the muscles of the leg and thorax or in the fatty tissue of the abdomen. The stylet is shed during or shortly after encystment, but persists and can be seen embedded in the cyst wall or partly disintegrated in the cystic fluid of even the oldest metacercariae. Under laboratory conditions at room temperature, the metacercariae develop in five weeks to the stage shown in Fig. 6, and attain a diameter of 0.18 to 0.25. At this time, the body begins to clear of the refractile globules that appeared soon after encystment and rendered it opaque. As the metacercariae age, the penetration glands and Virgula organ disappear and the digestive system becomes distinct. A very short prepharynx is present and the intestinal ceca end at a level with or slightly anterior to the ventral sucker. The excretory bladder promises to be U-shaped in the adult and is filled with highly refractile granules. Other details of internal structure were not distinct.

For experimental work, May-fly naiads were used, because their small size and



transparency made it possible to detect metacercariae through the body wall when the live insect was examined with the dissecting microscope. High mortality of infected naiads, however, greatly limited attempts to complete the life history.

The only complete life history reported for the *Virgula* group is that of *Loxogenes liberum*, traced by Okabe (1937). Since species of *Loxogenes* occur in frogs, it seemed advisable to test them as possible definitive hosts for the adult of *C. nyxetica*. First, an attempt was made early in the summer of 1944 to determine whether frogs collected in the vicinity of the snail host harbored an adult trematode that might be the one in question. Frogs were rare in the stream, only one being captured after careful search. Examination of this frog revealed the presence of a single, large specimen of *Loxogenes bicolor* Krull in an hepaticoduodenal cyst. An attempt was then made to determine whether *C. nyxetica* might be the larva of *L. bicolor* by feeding infected May-fly naiads to two frogs of a lot obtained from a commercial source outside the state. Several of the lot had been used previously for other purposes and found to be free of species of *Loxogenes*.

One of the experimental frogs was killed and examined five days after being fed naiads containing 40-day-old metacercariae and was found to be negative for any sort of trematode. The same results were obtained when the second frog was sacrificed several days later. Scarcity of materials and transportation difficulties prevented further attempts to infect frogs during the season of 1944.

There are few snails of any sort in McCormicks Creek except *Goniobasis depygis*, the host of *C. nyxetica*. The creek is rocky, small, and rapid with few pools of any considerable depth and thorough collecting is easily accomplished. Thousands of *G. depygis* and as many other snails as could be found have been collected from McCormicks Creek over a period of nine years and only one species of *Virgula* type cercariae has ever been discovered from that locality. It therefore seems likely that this species may be the larva of *L. bicolor* which certainly is known to occur in frogs of the vicinity, even though feeding experiments were negative. Since only two frogs were used and these were in poor condition because of prolonged captivity, the results obtained are considered to be inconclusive.

Description of *Cercaria neustica* sp. nov. (Figs. 10 and 11)

*Snail host*: *Pleurocera acuta* Rafinesque.

*Locality*: Eel River, North Manchester, Indiana.

*Incidence*: 1.0%.

*Specific diagnosis*: Maximal body length extended 0.23, minimum length contracted 0.08; body of moderately contracted specimens under light pressure averages 0.116 long, 0.065 wide, and 0.058 thick. Moderately contracted tail 0.07 long and 0.02 wide at the base. Oral sucker 0.03 in diameter, with relatively small *Virgula* organ 0.019 wide at the anterior end and 0.014 long. Stylet averages 0.023 long by 0.008 wide at the base, tip curved ventrally. Pharynx 0.012 long by 0.009 wide, esophagus and intestine not observed. Ventral sucker 0.019 in diameter and situated at approximately the middle of the body. Genital primordium 0.020 wide by 0.022 long, C-shaped and located at or slightly posterior to level of ventral sucker. Three pairs of cephalic glands, the most posterior having the coarsest cytoplasmic granules. Ducts of anterior pair separate from others, bending dorsad and then mediad before passing over the oral sucker. Flame cell pattern 2 [(2+2+2) + (2+2+2)]. Excretory bladder U-shaped and 0.023 long when fully distended. Develop in oval-round sporocysts in the digestive gland of the mollusk. No cysts were found in May-fly naiads after exposure for two weeks to a large number of cercariae.

Of the species described here, *C. neustica* was the only one in which the flame cell pattern could be seen with any degree of clarity. The main collecting tubule

of each side extends anteriorly a short distance and divides before reaching the ventral sucker to form a posterior and an anterior secondary tubule. Each secondary tubule receives the capillaries of six flame cells in groups of two, one dorsal and one ventral. The collecting tubules are seen clearly at rare intervals and, because the body is quite thick, the capillary connections of dorsal and ventral flame cells of each group are observed accurately only from the lateral aspect. The excretory system was obscured also by the refractile edges of what are presumably cystogenous glands just beneath the cuticle.

To the writer's knowledge, the complete flame cell pattern has been determined in only two other *Virgula* cercariae, viz., *C. helvetica* IX Dubois (1928) and *C. virguloides* Porter (1938). The pattern in the former species is apparently the same as that of *C. neustica*, and is one that has been described for several xiphidiocercariae of other types. The pattern described for *C. virguloides*, however, consisted of but eight pairs of flame cells arranged with two pairs on each of the anterior and posterior collecting tubules.

As in the case of the other species studied, the two antermost pairs of cephalic glands always discharge their contents before the posterior pair. The ducts of the posterior pair are very slender and follow a more or less tortuous path along the ducts of the second pair. In most specimens, the cytoplasm of the posterior pair stained more intensely with neutral red than did the others. Sometimes, however, all of the glands appeared the same color.

Description of *Cercaria meringura* sp. nov. (Figs. 1 and 2)

*Snail host:* *Goniobasis livescens* (Menke).

*Locality:* Tippecanoe River, Battle Ground, Indiana.

*Incidence:* 1.24%.

*Specific diagnosis:* Body length extended 0.22, contracted 0.1; moderately contracted body 0.13 long and 0.09 wide at level of ventral sucker. Tail extended approximately 0.16, contracted 0.045. Oral sucker 0.049 in diameter; stylet 0.046 long, 0.008 wide at base. *Virgula* 0.030 in diameter. Body spinose with more conspicuous spines around opening of ventral sucker. Tail aspinose except at tip which bears a patch of long spines. Body with papillae bearing fine setae as in other species reported here. Pharynx 0.015 long and 0.02 wide, often obscured by a mass resembling oil droplets; remainder of digestive system not observed. Genital primordium posterior to the ventral sucker. Three pairs of cephalic glands and ducts of each side passing forward in a single bundle and opening separately in a row close to stylet tip; anterior ends of ducts more distinct than in other species studied. Small bodies with the staining characteristics of the cephalic glands occur consistently in two pairs near the pharyngeal level and additional ones are sometimes present. Develop in oval-round or slightly irregular sporocysts in the digestive gland of the snail. Sporocysts measure up to 0.25 long and contain about a dozen cercariae. Attempts to induce penetration and encystment in May-fly naiads were unsuccessful.

Description of *Cercaria nothrica* sp. nov. (Figs. 8 and 9)

*Snail host:* *Pleurocera acuta* Rafinesque.

*Locality:* Tippecanoe River, Battle Ground, Indiana.

*Incidence:* 0.51%.

*Specific diagnosis:* Length of body extended 0.173, contracted 0.077; moderately contracted body 0.092 long and 0.073 wide at level of ventral sucker. Tail extended approximately 0.115 long, contracted 0.023 long and 0.023 wide at the base. Oral sucker 0.043 wide; stylet 0.022 long and 0.005 wide at base, tip curved ventrally. *Virgula* organ 0.030 long and 0.038 wide at the anterior end. Ventral sucker 0.020 wide, situated in a prominent bulge of the ventral surface. Body and tail spinose, longer spines on tail tip. Papillae with fine setae scattered over body. Pharynx 0.01 long; remainder of digestive system not observed. Genital primordium posterior to ventral sucker. Three pairs cephalic glands, the anterior pair with coarser granules than the others; neutral red stains posterior pair darkest. Cephalic gland ducts of each side pass anteriorly as a single bundle with separate openings near tip of stylet. Bladder U-shaped. Develop in oval-round sporocysts up to 0.16 long in digestive gland of snail.

Only two snails were found to harbor *C. nothrica* and both infections apparently were young, since only small numbers of cercariae emerged and most of the sporocysts obtained by cracking the snails were immature.

When not swimming, these cercariae habitually lie on their side or back, with the body strongly flexed, and extend and contract in a peculiar manner that distinguishes them from the other *Virgula* cercariae studied. Inch-worm movements were never observed in this species.

Description of *Cercaria tranoglandis* sp. nov. (Figs. 12 and 13)

*Snail host*: *Goniobasis livescens* (Menke).

*Locality*: Tippecanoe River, Battle Ground, Indiana.

*Specific diagnosis*: Body length fully extended 0.173, contracted 0.08; body at medium contraction 0.126 long and 0.076 wide at level of ventral sucker. Tail extended 0.144 long and 0.015 wide at base, contracted 0.036 long and 0.024 wide. Oral sucker 0.038 wide; stylet 0.022 long and 0.005 wide at the base, tip curved ventrally. *Virgula* organ 0.027 long and 0.027 wide at the anterior end. Ventral sucker in a conspicuous protrusion of the body and 0.016 in diameter. Body and tail finely spinose, spines longer on tip of tail. Papillae with delicate setae scattered over body. Pharynx 0.009 wide, remainder of digestive system not observed. Genital primordium 0.024 wide, C-shaped, slightly posterior to ventral sucker. Three pairs of cephalic glands, the anterior of which possesses very coarse granules. Posterior pair stains darkest with neutral red. Cephalic gland ducts of each side in a single bundle with individual openings at side of stylet tip. Two or more gland-like bodies similar to those of *C. meringura* usually present at pharyngeal level. Excretory bladder broad, U-shaped. Develop in oval-round sporocysts up to 0.2 long in the digestive gland of the snail host.

DISCUSSION

Minute differences between some of the cercariae reported here raises the question as to whether all of them are actually distinct species. The differences observed can be interpreted only as actual specific differences or variations with development in different host species. Life history studies have demonstrated that the cercariae of different species or even genera of adult trematodes may have larvae that are almost indistinguishable. Several examples that could be cited support the view that any constant differences between cercariae, however minute, should be regarded as of at least specific magnitude.

All five species of cercariae described here have the same number of cephalic glands, viz., three pairs. This characteristic immediately distinguishes them from *C. geddesi* Ameal, the only species of the *Virgula* type described from the U. S. Among the cercariae of that type occurring elsewhere, only the Indian species described by Sewell (1922) and *C. pyramidum* Azim (1936) possess three pairs of cephalic glands. Wesenburg-Lund's (1934) description of *C. nodulosa* von Linstow shows the same number, but with an additional pair of smaller median glands whose ducts do not empty at the stylet tip. Other *Virgula* cercariae are described as possessing four or more pairs.

The structure of the *Virgula* organ is essentially the same in all of the five present species, but differs from that described for certain others. Sewell noted a distinction between a bilobed organ with an aperture as in *C. indica* XLII and *C. virgula* Filippi, and a horseshoe-shaped structure that is hollow and with limbs passing backward and dorsally in the manner described for other *Virgula* cercariae. Since the five present species possess *Virgula* organs of the bilobed type, this characteristic distinguishes them and *C. indica* XLII from all other *Virgula* cercariae with three pairs of cephalic glands. From these six species, three, viz., *C. neustica*, *C. nothrica*

and *C. tranoglandis*, may be separated by the shape of the stylet, the tip of which is curved ventrally. *C. neustica* is peculiar in the small size of its Virgula organ and the medial path of the anterior cephalic gland duct. The chief differences between *C. nothrica* and *C. tranoglandis* are the presence of glandular bodies near the pharynx of *C. tranoglandis* and the more coarsely granular anterior cephalic glands of this species as compared with *C. nothrica*.

In contrast to the species differentiated above, *C. meringura*, *C. nyxetica* and presumably *C. indica* XLII possess straight stylets, since Sewell (1922) gives no indication to the contrary in his description of the last species. Of these three, *C. meringura* is unique in its large body size, disproportionately long stylet and presence of two pairs of glandular bodies near the pharynx. In *C. nyxetica*, the presence of a rhabdocoel gut, as described for *C. indica* XLII, could not be determined. However, the two are dissimilar in other respects. *C. nyxetica* is characterized by a knobbed stylet, single bundle of cephalic gland ducts on each side, linear cephalic gland arrangement and spinose tail, whereas in *C. indica* XLII, the stylet has only slight shoulders, one pair of cephalic glands and its ducts are mediad, and the tail is aspinose.

In view of the study of Okabe (1937), it seems likely that the Virgula cercariae are larvae of trematodes belonging to the family LECITHODENDRIIDAE. Observations on the metacercarial stages of *C. nyxetica* and ecological relationships of this larva to *Loxogenes bicolor* support this hypothesis.

The family LECITHODENDRIIDAE was created by Odhner (1911) to include two subfamilies, the LECITHODENDRIINAE Looss (1902) and PLEUROGENETINAE Looss (1899). Since that time, there has developed much difference of opinion concerning the intrafamilial relationships of the group, due to lack of agreement concerning taxonomic criteria such as the shape of the bladder, flame cell pattern and position of the vitellaria. A revision of the family was made by Mehra 1935 who reduced the family EUMEGACETIDAE Travassos to the rank of a sub-family and proposed the new sub-families PHANEROPSOLINAE, ANCHITREMINEAE, and EXITODENDRIINAE.

Life history studies in other families have cast serious doubt on the validity of many of the adult characters used as a basis for such attempts at classification as those mentioned above. For this reason and because of the lack of information concerning the life histories and embryology of members of the family LECITHODENDRIIDAE, it is believed that there is at present no adequate basis for proposing a natural and hence enduring scheme of classification within the family.

#### SUMMARY

Five new species of xiphidiocercariae of the Virgula type are described from Indiana, U. S. A. They are *Cercaria meringura* and *C. tranoglandis* from *Goniobasis livescens* (Menke); *C. nothrica* and *C. neustica* from *Pleurocera acuta* Rafinesque; and *C. nyxetica* from *Goniobasis depygis* (Say). A portion of the life history of *C. nyxetica* has been traced and lecitходendriid affinities indicated.

#### REFERENCES

- AMEEL, D. J. 1939 Cercariae infecting *Pomatiopsis lapidaria* Say. Am. Midl. Nat. 21(3): 651-656.  
AZIM, M. A. 1936 On the life history of *Lecithodendrium pyramidum* Looss, 1896, and its development from a xiphidiocercaria, *C. pyramidum* sp. nov., from *Melania tuberculata*. Ann. Trop. Med. Parasit. 30(3): 351-354.



- CABLE, R. M. 1939 Two new species of cotylomicrocercous cercariae from Indiana. Tr. Am. Micr. Soc. 58(1): 62-66.
- DUBOIS, G. 1928 Les cercaires de la région de Neuchâtel. Bull. Soc. Neuchât. Sci. Nat. 53: 1-177.
- KRULL, W. H. 1933 *Loxogenes bicolor*, a new pigmented fluke from the frog, *Rana clamitans* Latr. Tr. Am. Micr. Soc. 52(1): 47-50.
- LOOSS, A. 1899 Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus *Distomum* Retzius. Zool. Jahrbüch. Syst. 12: 581-784.
- 1902 Ueber neue und bekannte Trematoden aus Seeschildkröten. Zool. Jahrbüch. Syst. 16: 411-894.
- LÜHE, M. 1909 Parasitische Plattwürmer. 1. Trematoda. Süßwasserfauna Deutschlands. Heft 17, Jena.
- MEHRA, H. R. 1935 New trematodes of the family Lecithodendriidae Odhner 1911, with a discussion on the classification of the family. Proc. Acad. Sci. Agra and Oudh, India 5(1): 99-121.
- ODHNER, T. 1911 Zum natürlichen System der digenen Trematoden III, IV. Zool. Anz. 38: 97-117, 513-531.
- OKABE, K. 1937 On the life history of a frog trematode, *Loxogenes liberum* Seno. Annot. Zool. Japon. 16(1): 42-52.
- PORTER, A. 1938 The larval trematoda found in certain South African mollusca with special reference to schistosomiasis (*bilharsiasis*). Pub. S. Afr. Inst. Med. Res. 8: 492 pp.
- SEWELL, R. B. S. 1922 Cercariae indicae. Ind. J. Med. Res. 10(supplement): 370 pp.
- WESENBERG-LUND, C. 1934 Contributions to the development of the Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters. Danske Vidensk. Selsk. Skrifter, Naturv. Math., Ser. 9, 5(3): 223 pp.

## EXPLANATION OF PLATE I

All figures of entire cercariae are drawn to the same scale and stylets are represented at twice that magnification.

- FIG. 1. *Cercaria meringura*, ventral view.  
FIG. 2. Stylet of *C. meringura*, dorsal view.  
FIG. 3. *Cercaria nyxetica*, ventral view.  
FIG. 4. Stylet of *C. nyxetica*, dorsal view.  
FIG. 5. Young metacercaria of *C. nyxetica*.  
FIG. 6. Fully developed metacercaria of *C. nyxetica*.  
FIG. 7. Sporocyst of *C. nyxetica*.

## EXPLANATION OF PLATE II

All figures of entire cercariae are drawn to the same scale and stylets are represented at twice that magnification.

- FIG. 8. *Cercaria nothrica*, ventral view.  
FIG. 9. Stylet of *C. nothrica* in (a) lateral and (b) dorsal aspects.  
FIG. 10. *Cercaria neustica*, ventral view.  
FIG. 11. Stylet of *C. neustica* in (a) lateral and (b) dorsal aspects.  
FIG. 12. *Cercaria tranoglandis*, ventral view.  
FIG. 13. Stylet of *C. tranoglandis* in (a) dorsal and (b) lateral aspects.



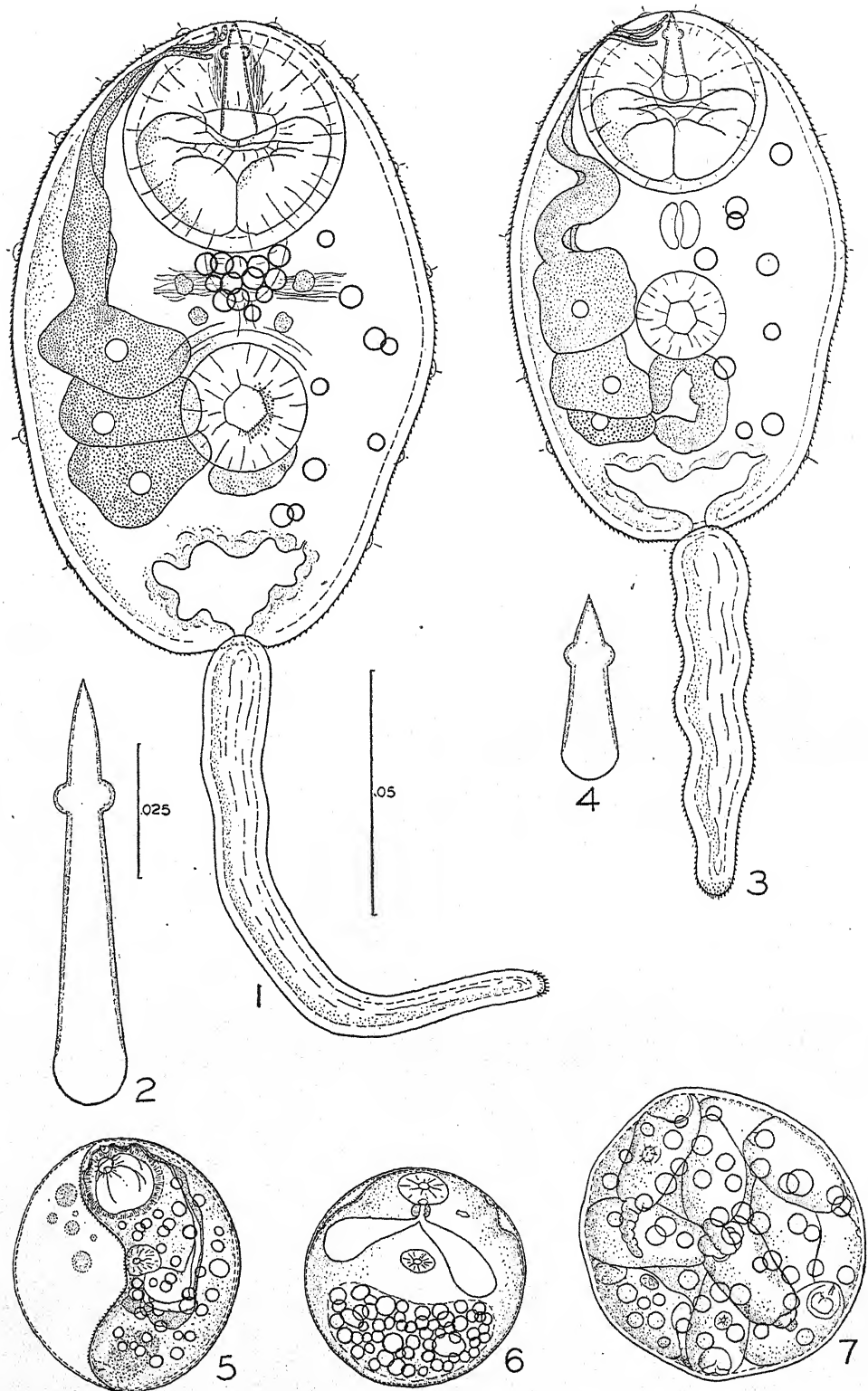


PLATE I

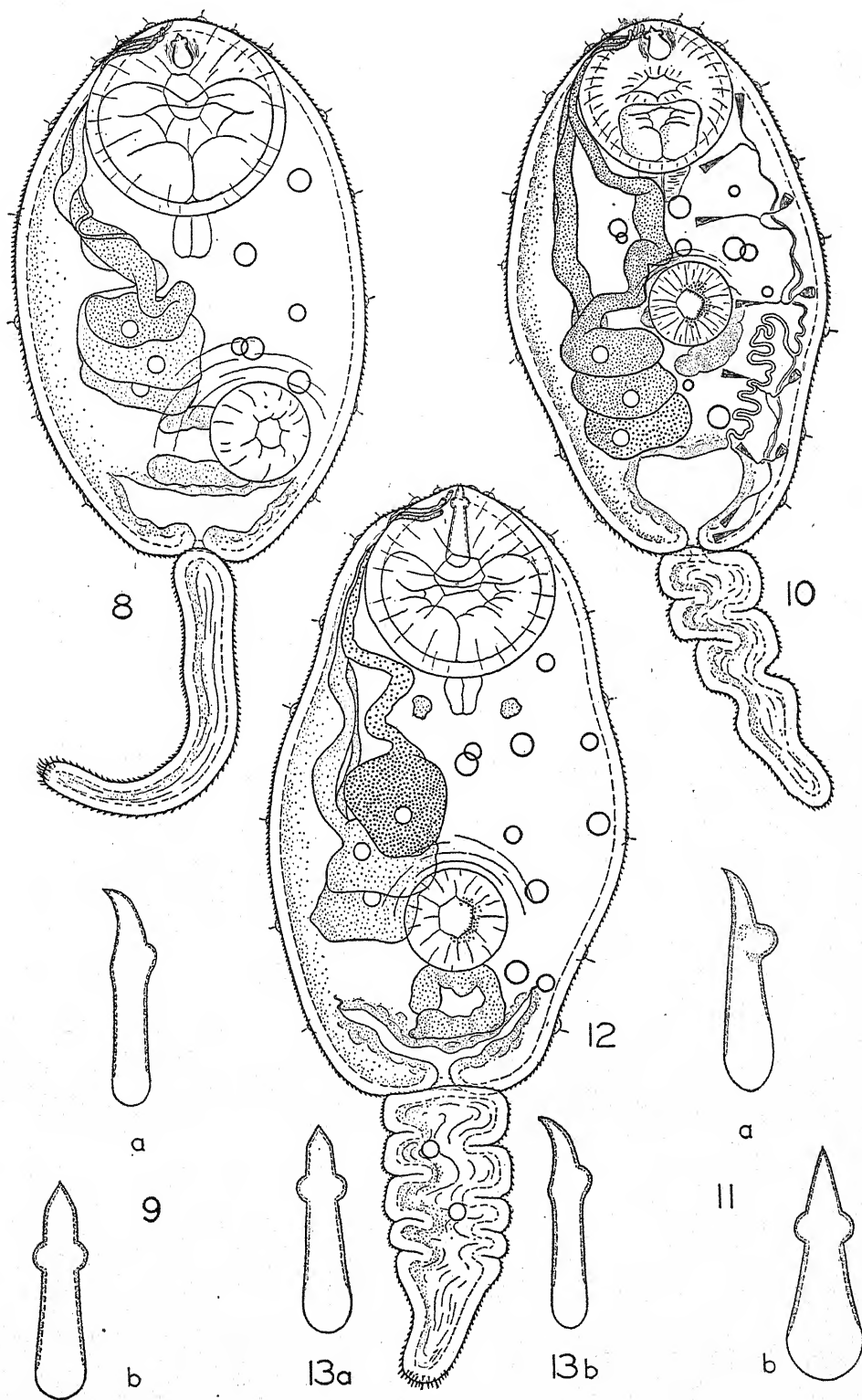


PLATE II

*TROMBICULA FRITTSI* N. SP. (ACARINIDA: TROMBICULIDAE)

LIEUT. (JG) G. W. WHARTON, H(S), USNR<sup>1</sup>

U. S. Naval Medical Research Unit No. 2<sup>2</sup>

Specimens of a new species of *Trombicula* were collected on Bougainville Island by J. M. Fritts, PhM2c, during the summer and fall of 1944. These specimens are described under the name *Trombicula frittsi*.

Womersley and Heaslip (1943) devised a series of standard measurements of the scutum. Their system is adopted and the designations of the measurements follow:

AW—distance between the bases of the anterior-lateral setae.

PW—distance between the bases of the posterior-lateral setae.

SB—distance between the centers of the pseudostigmata.

ASB—greatest perpendicular distance from a line drawn through the centers of the pseudostigmata to the anterior margin of the scutum.

PSB—greatest perpendicular distance from a line drawn through the centers of the pseudostigmata to the posterior margin of the scutum.

AP—distance between the bases of the anterior-lateral and posterior-lateral setae.

AM—length of the anterior-median seta.

AL—length of the anterior-lateral setae.

PL—length of the posterior-lateral setae.

S—length of the pseudostigmatic organs or sensillae.

DS—length of the dorsal setae.

All of the measurements are given in microns. While consideration of these standard data are extremely useful, careful allowances must be made for variations within species and unavoidable errors in measurement.

DESCRIPTION

*Trombicula frittsi* n. sp. (Figure 1). Only engorged larvae were collected. Length including capitulum, 400 microns. Maximum width, 250 microns. Color red. Striae on cuticle not prominent.

*Palps* (Figure 1, C and D): Segment 1 with a branched seta originating from the anterior-lateral region. Segment 2 rounded laterally, seta with not more than two delicate branches. Segment 3 with a nude seta. Segment 4 with nude dorsal and lateral setae, ventral seta with two lateral branches. Segment 5 with one proximal-ventral sensory seta, one medial-ventral branched seta, one central-ventral nude seta, one lateral nude seta, and two heavily barbed apical setae. Palpal claw trifurcate. Cheliceral shield well developed with one pair of nude setae.

*Chelicerae* (Figure 1, C and D): Basal segment evenly rounded, 20 microns long, 15 microns wide, and lightly ornamented with small pits. Distal chitinous segment strongly curved with a single recurved dorsal tooth and a single recurved ventral tooth. Apex sharp dorsally, rounded ventrally.

*Legs* (Figure 1, A and B): Coxae each with a single branched seta. One sensory seta on each tarsus. Tibia III with a sensory seta.

*Scutum* (Figure 1, E): Pentagonal with a few scattered pits. Anterior angles smallest, posterior-median angle greatest. Posterior margin folded at its apex. Setae all with short barbs. Pseudostigmatic organs filiform with short lateral branches on the distal two-thirds. Standard measurements in microns:

AW—43, PW—56, SB—17, ASB—17, PSB—21, AP—18, AM—22, AL—21, PL—29, S—39, DS—22-26.

Received for publication, June 1, 1945.

<sup>1</sup> On military leave from Duke University.

<sup>2</sup> The opinions expressed are the author's and not necessarily those of the Navy Department.

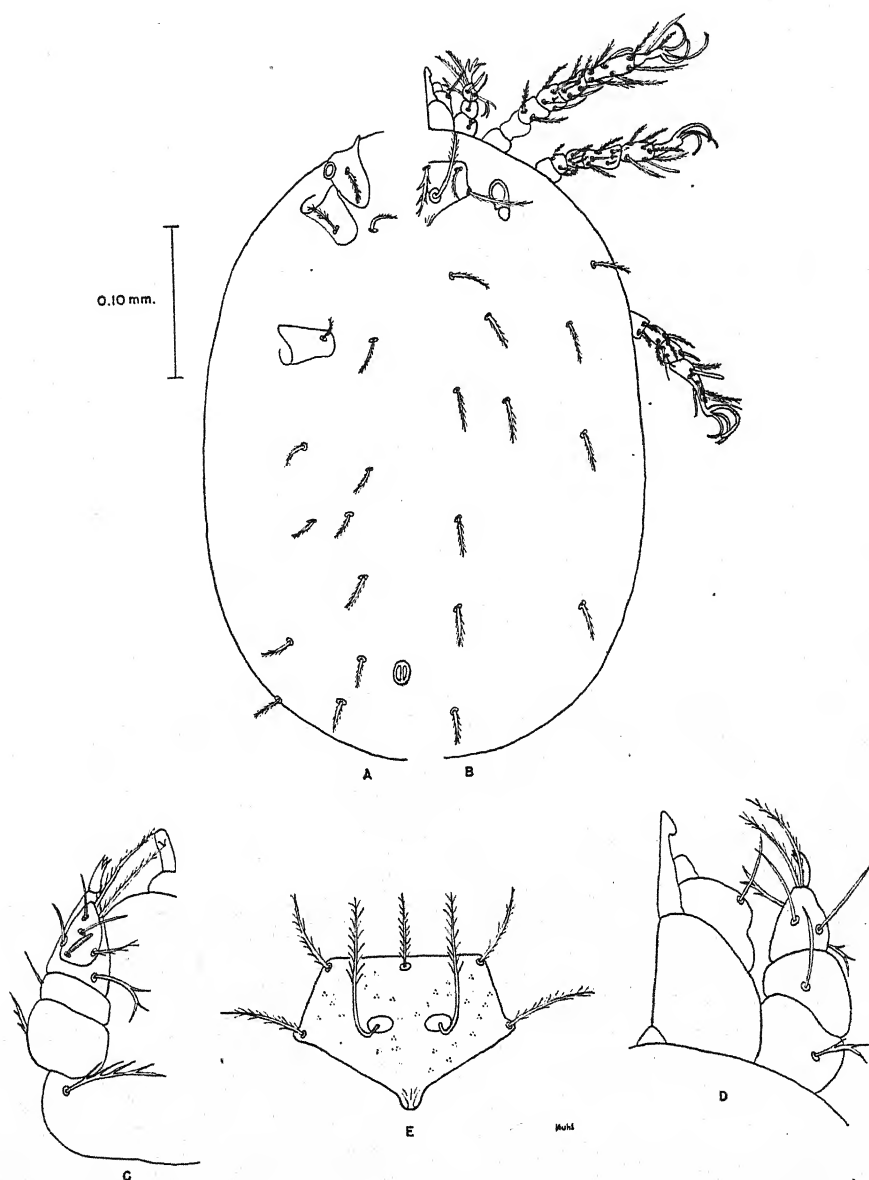


FIG. 1. *Trombicula frittsi* n. sp. A, ventral view; B, dorsal view; C, ventral view of capitulum  $\times 1000$ ; D, dorsal view of capitulum  $\times 1000$ ; E, scutum  $\times 500$ .

*Setae*: Dorsal formula, 2-6-6-4-2-2-; ventral formula, 2-2-4-4-4-4-2. Dorsal and ventral setae similar in shape, all with short lateral barbs.

*Hosts*: *Gehyra oceanica*, *Varanus indicus*, and *Rattus praetor*.

*Locality*: Cape Torokina, Empress Augusta Bay, Bougainville Island, Australian Mandate.

*Type*: U. S. National Museum.

*Paratypes*: U. S. National Museum; South Australian Museum.

*Diagnosis*.—*Trombicula frittsi* can be readily distinguished from all other members of the genus by the ridges and folds on the scutum at its posterior-median angle.

#### REFERENCE

- WOMERSLEY, H. AND HEASLIP, W. G. 1943 The Trombiculinae (Acarina) or itch-mites of the Austro-Malayan and Oriental regions. Trans. Roy. Soc. South Australia 67: 68-142.

## TRICHURIS SPECIES FROM CALIFORNIA RODENTS

ASA C. CHANDLER

Biological Laboratory, Rice Institute, Houston, Texas

Study of a collection of whipworms obtained from California rodents collected by the Hastings Natural History Reservation near Monterey, California, revealed the presence of four species, three of which are new, and indicates a marked host specificity for species of this genus. Descriptions of the new species follow, and also some additions to Hall's (1916) description of *T. fossor*.

### *Trichuris fossor* Hall, 1916

Specimens from *Thomomys bottae bottae* are larger than those described by Hall, 1916, from *Thomomys fossor* in Colorado, the males measuring 28 to 30 mm in length, and the females 28 to 33 mm. In Hall's specimens, which were probably immature, the anterior portion of the body exceeded the posterior portion in length, but in my mature specimens the posterior portion equals or exceeds the anterior portion in length. The specimens agree in the bilobed end of the male body, the bell-shaped, spiny, spicular sheath, and the length of the spicule (about 1.5 mm long in my specimens). The spicule flares slightly a short distance behind the tip. The cloacal tube is about 2.5 mm long, and is joined by the spicular tube near the middle of its length. The ejaculatory duct joins the vas deferens at about the middle of the length of the posterior portion of the body. In the female the vulva is not situated on a prominence, and the ovejector is from 1.1 to 2 mm long, gradually widening into the uterus. The anus is subterminal. The eggs, not seen by Hall, are relatively broad, measuring 64 to 68 microns by 35 to 38 microns.

### *Trichuris citelli*, n. sp.

*Description:* Male 34.5 to 38.5 mm long; posterior part of body 12.5 to 14.5 mm long, anterior part 21.5 to 24.5 mm long, the ratio being 3:5 to 4:7. Maximum diameter of posterior part about 600 microns; diameter at end of esophagus about 350 microns; diameter of anterior part of body gradually increasing from 75 to 200 microns. Posterior end of body bilobed. Spicule 1.3 to 2.1 mm long, tapering in its proximal two-thirds from a diameter of 75 to 100 microns to about 45 microns, then maintaining this diameter to about 60 microns of distal end, where it abruptly narrows to a conical tip. Spicule sheath, when fully evaginated, projecting from body 270 to 360 microns, the exerted part forming a bulb about 300 microns broad, with the spines developed best on its proximal surface. Cloacal tube (cloaca to junction of ejaculatory duct and intestine) 1.5 to 1.9 mm long, spicular tube joining it about 1 to 1.3 mm from posterior end. Junction of ejaculatory duct and vas deferens 6.5 to 8.5 mm from posterior end of body. Vas deferens 5 to 6 mm long, looping back to form testis just behind end of esophagus. Testis lobulated, lobes 800 to 900 microns long posteriorly, 150 to 450 microns long near middle and anteriorly. Testis ends about at proximal end of cloacal tube.

Female about 45 mm long; posterior part of body 18 to 19.5 mm long, anterior part 26 to 27.5 mm long, the ratio being 2:3 to 3:4. Vulva situated on a prominence, approximately at end of esophagus. Maximum diameter of posterior part of body 700 microns, of anterior portion increasing from 100 to 200 microns; diameter at vulva 350 to 400 microns. Ovejector 1.3 to 1.6 mm long, enlarged into a chamber at posterior end. Uterus loops back to form ovary about 1.5 to 2 mm from posterior end. Rectum 225 to 275 microns long. Eggs 70 to 74 microns by 33 to 35 microns.

*Location:* Caecum.

*Host:* *Citellus beecheyi*.

*Locality:* Hastings Natural History Reservation, California.

*Type and allotype:* U. S. National Museum, Helm. Coll. Nos. 45,824, 45,825.

Received for publication, June 2, 1945.



This species resembles *T. muris* (Schrank, 1788) Hall, 1916, as redescribed by Cerecero, 1943, in many respects, but is much larger, with a much longer and stouter spicule and larger eggs. In *T. muris* the proximal end of the cloacal tube is about one-third the distance from the posterior end of the body to the junction of the ejaculatory duct and vas deferens, whereas in *T. citelli* it is about one-fourth of this distance.

*Trichuris perognathi*

*Description:* Male 25 to 30 mm long, posterior part of body approximately same length as esophageal part. Maximum diameter of thick part about 350 microns, of esophageal part about 90 microns. Spicule 0.9 to 1.15 mm long, 20 microns in diameter near the rounded tip, flaring to diameter of about 45 microns about 250 to 300 microns from tip, then narrowing somewhat, and broadening again at proximal end to diameter of about 50 microns. Spicule sheath bell-shaped when exerted, covered with dense, blunt spines on terminal 220 to 300 microns. Spicular tube joins cloacal tube about 400 microns from posterior end. Cloacal tube about 2 mm long. Junction of ejaculatory duct and vas deferens not observed. Testes irregularly lobulated.

Female 46 to 47 mm long, the esophageal portion equal to or slightly shorter than thick portion of body (21 to 23 mm long). Maximum diameter about 550 microns, near middle of thick part of body. Body tapers markedly posteriorly, and ends in a truncated tip about 150 microns in diameter. Vulva situated just posterior to end of esophagus, and marked by a backward projecting cuticular evagination about 100 microns long. Ovejector about 0.8 to 1.1 mm long, widening abruptly into the uterus. Eggs narrow, 65 to 67 microns by 31 to 33 microns.

*Location:* Caecum.

*Host:* *Perognathus californicus californicus*.

*Locality:* Hastings Natural History Reservation, California.

*Type and allotype:* U. S. National Museum, Helm. Coll. Nos. 45,828, 45,829.

This species is readily distinguished by the backward projecting cuticular evagination on which the vulva is situated, by the tapering of the posterior end of the body of the female, by the failure of the esophageal portion of the body to exceed the posterior portion in length in either sex, and by the characters of the spicule and its sheath in the male.

*Trichuris neotomae*

*Description:* Male 22 to 23 mm long, posterior part of body 7.5 to 8 mm long, anterior part 14 to 17 mm long, the ratio being 1:2 to 3:5. Maximum diameter of posterior part 475 to 500 microns, diameter of anterior part about 140 microns proximally, tapering to about 125 microns in cephalic half. End of body distinctly bilobed, the indentation between the lobes being about 50 to 60 microns deep. Spicule 1.15 to 1.23 mm long and 30 microns wide near tip, flaring to about 45 microns about 0.5 mm from proximal end. Spicular sheath, when fully evaginated, protrudes from body about 440 microns, the protruded part with an expanded tip 137 microns broad and 155 microns long, then narrowing to a diameter of 65 microns, the entire extruded part covered with spines. Inside body, cloacal tube heavily chitinized and corrugated for about 265 microns, then widening to a still more heavily chitinized but less markedly corrugated chamber 200 to 250 microns long and 100 microns wide, ending at point where spicular tube and cloacal tube join, about 570 to 600 microns from posterior end of body. Rest of spicular tube with thin walls. Junction of spicular tube and cloacal tube to proximal end of cloacal tube about 660 to 700 microns, entire cloacal tube being, therefore, about 1.2 to 1.25 mm long. Ejaculatory duct about 3 mm long, and vas deferens about 3.75 mm long, the two joined by short, narrow duct. Testis irregularly lobulated, extending from posterior end of esophagus to proximal end of cloacal tube.

Female 28 to 34 mm long; posterior part of body 13 to 16 mm long, anterior part 16 to 21 mm long, the vulva at posterior end of esophagus, dividing body approximately 1:1.25 to 1.6. Vulva situated on a slight prominence. Maximum diameter of posterior part of body 700 to 800 microns, of anterior part 150 to 180 microns. Ovejector about 1 to 1.4 mm long, with space for only a single row of eggs. Anus 30 to 40 microns from posterior end, which is bluntly rounded. Eggs long and narrow, measuring about 90 by 40 microns.

*Location:* Caecum.

*Host:* *Neotoma fuscipes*.

*Locality:* Hastings Natural History Reservation, California.

*Type and allotype:* U. S. National Museum, Helm. Coll. Nos. 45,826, 45,827.

This species resembles *T. muris* as described by Cerecero (1943) in most respects, but differs in the greater length and stoutness of the spicule, the longer ovejector, and the much larger eggs. It is most strikingly characterized by the heavily chitinated and corrugated walls of the cloacal tube distal to the junction of the spicular tube.

## SUMMARY

*Trichuris fossor* is reported from a new host, *Thomomys bottae bottae*, and three new species of *Trichuris* are described: *T. citelli* from *citellus beecheyi*, *T. neotomae* from *Neotoma fuscipes*, and *T. perognathi* from *Perognathus californicus californicus*.

## REFERENCES

- CERECERO, D., MARIA C. 1943 Algunos Helmintos de las Ratas Domesticas y Silvestres de Mexico. Tesis, Universidad Nac. Auton. de Mex., Facultad de Ciencias, Mexico.  
HALL, M. C. 1916 Nematode Parasites of Mammals of the Orders Rodentia, Lagomorpha and Hyracoidea. Proc. U. S. Nat. Mus. 50: No. 2131, pp. 1-258.

## HELMINTHS FROM THE BOB-WHITE QUAIL IN TEXAS

J. DAN WEBSTER\* AND C. J. ADDIS

Biological Laboratory, Rice Institute, Houston, Texas

From 1941 to 1943, in cooperation with several men of the Texas Game, Fish and Oyster Commission, we examined 290 Texas Bob-white Quail (*Colinus virginianus texanus*) for helminth parasites, under the supervision of Dr. Asa C. Chandler. The majority of the examinations was made by the junior author; the senior author is responsible for the identifications. Actually, only the viscera were examined of all of the 290 birds; also tracheae of 55 and entire bodies of 12 were available. The quail were taken by Valgene W. Lehmann, W. Garner Fuller, Rollin H. Baker, J. M. Carlisle, and the senior writer. 276 birds came from South Texas (Jim Hogg, Jim Wells, Duval, and Zapata Counties) and 14 from Central Texas (Coleman, Harris, Trinity, and Colorado Counties). All of the birds were full-grown except for six downy chicks. It is suggested that comparison be made with the work of Cram, Jones, and Allen (1936).

## CESTODES FOUND

*Raillietina* (*Raillietina*) *colinia* Webster, 1944, was encountered only once (Webster, 1944), in September from Central Texas.

*Raillietina* (*Skrjabinia*) *cesticillus* (Molin, 1858) Joyeux, 1923, was taken only once (Webster, 1944), a heavy infestation mixed with the preceding species.

*Paricterotaenia* sp., was encountered once—a single worm from South Texas in December.

*Rhabdometra odiosa* (Leidy, 1887) Jones, 1929, was found in 14 quail from South Texas, not more than seven worms in any one infestation. There was 19% infestation in 47 quail taken in South Texas from May to August, but only 2% infestation in 205 birds taken there in December and January.

Received for publication, April 30, 1945.

\* Now 1st Lieutenant, Sanitary Corps, Army of the United States.

## NEMATODES FOUND

*Aulonocephalus lindquisti* Chandler, 1935, has previously been reported only in the original description (Chandler, 1935), which was based on specimens from a Scaled Quail (*Callipepla squamata*) taken at Uvalde, only 150 miles northwest of the origin of most of the present records. This nematode has a geographical correlation; of 14 quail examined from Central Texas there was only one record, as compared with 92% infestation of the 270 adult quail from South Texas. The worms were commonest in the caeca although they frequently occurred in the large intestine; mature males varied from 8 to 10.6 mm in length; mature females varied from 10 to 14.5 mm in length. One difference in anatomy was found as compared with Chandler's description: position of the vulva was found to be slightly anterior to the middle of the body, dividing the body in a ratio of from 10:11 to 10:13 (fifteen females measured, from fifteen hosts), whereas Chandler reported that the vulva in his three female specimens was at, or slightly posterior to, the middle of the body. As many as 300 worms were taken from a single host, but 15 to 40 was most common.

*Cyrnea* sp. occurred but once; two males were taken from a proventricular lining in June from South Texas.

*Syngamus trachea* (Montagu, 1811) Chapin, 1925, was taken twice, in summer from South Texas.

## REFERENCES

- CHANDLER, A. C. 1935 A new genus and species of Subularinae (Nematodes). Tr. Am. Micr. Soc. 54: 33-35.  
CRAM, E. B., JONES, M. F. AND ALLEN, E. A. 1936 Chap. IX in Stoddard, H. L. The Bob-white Quail; Its habits, preservation and increase. Charles Scribner's Sons, New York.  
WEBSTER, J. D. 1944 A new cestode from the Bob-white. Tr. Am. Micr. Soc. 63: 44-45.

## RESEARCH NOTES

### PINWORM INFESTATION AMONG CHILDREN OF RURAL COMMUNITIES

During the winter of 1942-43, 315 school and preschool children, residents of several small towns in southwestern South Dakota, were examined for pinworm ova. The method used was that described by Jacobs (1942, *Journal Pediatrics* 21: 497), i.e., a piece of Scotch tape held over a tongue depressor with adhesive side out was applied to the perianal folds, flattened on a glass slide, and examined microscopically. Each child who was examined had to submit a filled-in questionnaire which included a written request from his parent. The parent was asked to state (yes or no when possible) whether the child slept well, ate well, was "nervous" in his opinion, was bothered by rectal itching, had many stomachaches or similar pains. Each child was weighed and measured at time of examination. With occasional exceptions, each child was examined but once.

Of the 315 children, 124 or 39.4% were positive for pinworm ova. This percentage would doubtless have been higher had repeated examinations been made. As it is, it compares closely with reported incidences among urban and institutionalized children. 36.2% of the boys who were examined (59 of 163), and 42.8% of the girls (65 of 152), were positive. The distribution of infection according to ages was as follows:

Age at last birthday	No. pos.	No. examined	% pos.
Pre-school: 1 through 5	11	44	25
School:			
6	20	36	56
7	16	37	43
8	12	40	30
9	19	37	43
10	22	46	48
11	9	36	25
12	13	24	54
13 through 15	2	15	13
Total	124	315	39.4

A sharp rise in rate of infestation was apparently coincident with attendance at school.

Information elicited from the parents showed the following differences—or similarities—between children found to be infected and children who upon one examination were negative for pinworm ova:

	Among 191 "negative" children	Among 124 "positive" children
No complaints	45%	35%
Complaints:		
1) anal pruritus	15%	27%
2) "nervousness"	36%	42%
3) underweight	17%	15%
4) insomnia	17%	9%
5) anorexia	15%	16%

Since the true incidence of infestation was not determined, undue significance cannot be attached to these figures. They suggest, however, that the frequent occurrence of symptoms among infested children should not be assumed to be associated with the infestation unless comparison with a similar uninfested group shows that the assumption is warranted.—EVELYN A. MAUSS, *Division of Public Health Laboratories, State Board of Health, Rapid City, South Dakota* in cooperation with the Fall River-Custer Counties Health Dept. and with the school authorities of those counties.)

### CHILLING AS A MEANS OF RETAINING THE VIABILITY OF THE SPOROZOITES OF *PLASMODIUM GALLINACEUM*

In sporozite-induced *Plasmodium gallinaceum* infections the procedure of exposing chicks to the bites of single infected *Aedes aegypti* mosquitoes is time-consuming and results in infections in only 85 per cent of the birds according to Coatney et al (1945 *Am. J. Hyg.* 41: 109). Later, Coatney et al (1945 *Am. J. Hyg.* 41: 119), reported infections in only 97.5 per cent of

200 birds into which infected isolated salivary glands had been injected subcutaneously. For many purposes, for example drug testing, it is desirable to avoid any uninfected birds in an experiment. It has been our experience, as well as that of others (Coatney et al 1945, Am. J. Hyg. 41: 119; personal communication with R. J. Porter) that the injection of pooled sporozoite suspensions invariably results in an infection.

Needless to say, it is also desirable to have uniform infections throughout an experiment. Since the viability of sporozoite suspensions may be adversely affected by unfavorable conditions, attempts have been made to improve the preparation and use of these suspensions. To reduce the time required for the preparation of the infective material, whole mosquitoes (etherized less than 1 minute) are ground in a Ten Broeck tissue grinder (Scientific Glass Co.) for less than 5 minutes. Porter (1942, J. Inf. Dis. 71: 1) indicated suspensions prepared in normal saline were irregularly infective, therefore normal chicken serum was used. To remove larger particles of chitin, etc., the material is strained three times through several layers of gauze. In each experiment, two control groups of 5 birds each are used; the first control group inoculated at the beginning and the second at the end of a series of test groups.

When kept at room temperature the viability of the sporozoites decreases during the period of about an hour required to inoculate intravenously 200 to 300 chickens. This is evidenced by the significant difference in parasitemia on the eighth day of infection. For example, in four separate tests the average parasitemia of the terminal control group in a series of 200 birds expressed as a percentage was only 10, 23, 23 and 11.6 per cent of the average parasitemia of the initial control group. Dr. E. Waletzky of this laboratory suggested that sporozoites might retain their viability to a greater extent if the ground mosquito material was chilled as soon as possible and kept in an ice bath during the period of inoculation. When such a procedure was used a more uniform infection was obtained since corresponding percentages for five separate trials were as follows: 71, 52, 77, 63 and 91.

The sparing effect of low temperature is also indicated by the increased virulence of chilled preparations. In 11 separate tests using an inoculum per bird of the unchilled sporozoites from about one mosquito, the average parasitemia on the eighth day of the infection for the first control group varied from 3 per cent to 48 per cent with a mean of 22.17 and a standard deviation of 14.1. The first trial using about the same size inoculum of chilled sporozoites resulted in the death of 3 of the 5 birds in the first group on the seventh day of the infection and parasitemias in the remaining two birds of 38 and 68 per cent. The second trial resulted in the death of all 5 birds on or slightly before the seventh day of the infection. Because of the apparent increase in the virulence of the sporozoites only about one-half mosquito was used for each inoculum in the third trial. This dose resulted in an average parasitemia of 44 per cent on the seventh day. In five subsequent trials the inoculum was reduced to consist of sporozoites of about one-tenth of a mosquito. The mean parasitemia of 4 of the first control groups was 36, 34, 34 and 13 per cent. In the fifth test 3 of the 5 birds died on the seventh day and the parasitemia of the remaining 2 was 45 and 51 per cent on the eighth day. Oöcyst counts indicate that the increased virulence seen following the adoption of the procedure of chilling the sporozoites cannot be accounted for by any significant increase in the intensity of the infections in the mosquitoes. This increase in virulence of the standard inoculum due to chilling was unexpected and suggests that even as short an exposure as 15 minutes (the maximum time intervening between the preparation and use of an inoculum) to room temperature results in marked injury to sporozoites.

*Summary:* Chilling pooled sporozoite material results in more uniform infections throughout a series of animals in the same experiment.—STERLING BRACKETT AND CARRIE OLA HUGHES, *Stamford Research Laboratories of The American Cyanamid Company, Stamford, Conn.*

#### CONFLICTING VIEWS IN REGARD TO *IODAMOEBIA WILLIAMSII*

It is frequently stated in text-books, that cysts of *Iodamoeba williamsii* are commonly seen in human stools, but that trophozoites of the same species are quite rare.

In a recent parasitology manual, by a well-known American author, the contrary statement is made, i.e., that vegetative forms are fairly common in human stools, but that cysts are rare.

This last statement, which I suppose was only a slip of the pen, already corrected in a second printing, called my attention to the need of verifying the correctness of the contrary assumption, which is the one generally held.

As there were in our files the results of a number of fecal examinations, in which we have carefully noted the instances when only cysts were found, only trophozoites, or both, of the different human intestinal protozoa, I thought it might be of some interest to record such results.

The groups providing these data are two. First group (A) comprised 100 purged fecal samples, positives for *I. williamsii*, examined in fresh saline and iodine smears, as well as in hematoxylin-stained slides; a second group (B) comprises 100 normally passed fecal samples,



positive for *I. williamsi*, examined in fresh saline and iodine smears, as well as in iodine smears after zinc sulphate concentration.

The results are as follows:

	(A)	(B)
Number of infections .....	100	100
Only cysts were found .....	32	67
Only trophozoites .....	32	16
Both cysts and trophozoites .....	36	17

On the basis of the above data, I believe that, in purged fecal samples, without concentration methods, when not only fresh examinations, but also hematoxylin-stained smears, which greatly help in doubtful cases, are made, the chances of finding trophozoites or cysts of this species, are almost equal. When formed stools are examined, without hematoxylin-stained smears, but using the zinc sulphate concentration method, the chance of finding only cysts increases as may be theoretically expected; but even in these conditions, trophozoites alone, or together with cysts, may be found in 33% of the infections, which hardly justifies the statement that they are "rarely" or "very rarely" seen.

I believe Wenyon's statement (Protozoology, 1926, Vol. I, p. 246) that "A remarkable feature of the infections is that often enormous numbers of cysts are passed without there being any indication of the free forms," with the weight given to it not only by the well-known competence of the author, who was one of the first to call attention to the "iodine bodies," but also from its inclusion in one of the best available protozoology text-books, has only been passively followed and rewritten by most authors.

It is my personal opinion that calling the students' attention, as many text-books do, to the fact that trophozoites of *Idodamoeba williamsi* are very rarely seen, results in their frequently overlooking the vegetative forms. The low incidence of this species reported in many surveys, in different localities, may perhaps be explained on such grounds.—ENRIQUE BELTRÁN, *Institute of Public Health and Tropical Diseases (México, D. F.)*.

# The Journal of Parasitology

Volume 31

OCTOBER, 1945

Number 5

## EFFECTS OF ALCOHOL ON NATURAL RESISTANCE TO THE DWARF TAPEWORM IN MICE<sup>1</sup>

JOHN E. LARSH, JR.

Mice have a strong natural resistance to infection with the dwarf tapeworm, *Hymenolepis nana* var. *fraterna*. Following initial infection it is common for less than five per cent of the eggs to develop into mature cysticercoids in the intestinal villi (Hunninen, 1935; Larsh, 1943b). The underlying mechanism responsible for this resistance is undetermined, but certain factors as age, concurrent infection and splenectomy have been shown to have a favorable or unfavorable influence. The present study was an attempt to gather additional information by determining the influence of alcohol on this resistance. Tests with the drug were performed: (1) to determine the effect on resistance of various concentrations (10 to 45 per cent) given for varying periods of time; (2) to compare the effect on resistance of injections given orally with those given by peritoneum; (3) to learn whether or not alcoholic debilitation is permanent; and (4) to determine the effect on resistance of one intoxicating dose.

Many workers have found large amounts of alcohol detrimental to the process of resistance to many bacterial infections. This is well illustrated by the work of Koch (1885), Doyen (1885) and Thomas (1893) on cholera; of Laitinen (1900) and Goldberg (1901) on anthrax; and of Rubin (1904) and Pickrell (1938) on pneumococcal infections. In short, numerous investigators using many different infecting agents agree that alcohol in large amounts, especially over long periods, has a definitely harmful effect on the natural defenses of many animals. Although the effect of moderate amounts of alcohol is disputed, most workers believe that small quantities (less than 1.5 cc per kilogram) have little or no effect on resistance (Laitinen, 1900; Kruschilin, 1909; Parkinson, 1909). From a review of experimental work, one concludes that the effect of alcohol on the bodily processes involved in resistance depends in large measure on the concentration of the drug and the number of injections given.

### METHODS

The mice, of the same strain used in earlier work, were raised *Hymenolepis*-free in this laboratory in an air-conditioned room maintained at 75° F. Ninety-five per cent alcohol was diluted with distilled water to obtain the percentage by volume of the drug needed. When given by way of the gastro-intestinal tract the alcohol was administered into the esophagus through a blunt 18-gauge needle at

Received for publication, April 6, 1945.

<sup>1</sup> Contribution from the School of Public Health, University of North Carolina, Chapel Hill, North Carolina.

tached to a one-cubic centimeter syringe. In all cases, the doses were confined in a volume of 0.4 cc. Intraperitoneal injections were given aseptically using a similar syringe and a 27-gauge needle.

In preliminary tests on mice 2-3 months old, it was found that concentrations of 50 per cent or more by mouth were very toxic and the survival rate low, but a high percentage survived doses of 45 per cent given daily for one month. Of those failing to survive, females outnumbered the males two to one. This amount of alcohol produced intoxication (unconsciousness) within ten minutes and stupor lasted two to six hours. It often was necessary to separate animals after being injected to prevent destruction of those that recovered slowly. After recovery most of them showed no noticeable variation from controls in physical appearance or behavior. Others dropped a few grams in weight, developed diarrhea, and the coat lost its natural gloss and became quite ruffled. Concentrations of 40 and 35 per cent given in the same volume to different mice of the same age produced intoxication almost as rapidly as 45 per cent and the animals required about the same length of time to recover. However, 25 per cent alcohol in most cases did not produce narcosis, but the animals staggered about in a stuporous state for an hour or more. Concentrations below 25 per cent given in the volume indicated had no visible effect on most of the animals.

Preliminary tests also were made of young mice given normal saline in place of alcohol once daily for several weeks to determine whether or not the injections alone produced mucosal damage which resulted in a higher percentage development of cysticercoids. After infection the number of parasites was about the same as in untreated controls so the procedure was omitted in the experiments reported below.

The methods of obtaining eggs and calculating doses for infections, of preparing the intestine free of mucus to facilitate cysticercoid counting, and other experimental details can be referred to elsewhere (Hunninen, 1935). As in previous work, all cysticercoid counts were made of animals killed 93 hours after infection. The  $M_2$  strain of the parasite was used, but eggs for infections were not stored as in recent work (Larsh, 1943b).

#### EXPERIMENTAL RESULTS

1. *The effect on natural resistance of twenty daily injections of 45 per cent alcohol.*—Experimental mice two months old were matched with controls of similar age, sex, and approximate weight. Each of the experimental animals was given by mouth a daily injection of 0.4 cc of 45 per cent alcohol for a period of 20 days. On the basis of the preliminary tests mentioned above, this represented a maximum sublethal amount. Twenty-four hours after the last dose, each of the alcoholic mice and their controls was infected with 900 *Hymenolepis* eggs. The number of cysticercoids counted in each mouse and the calculated percentage development is given in Table 1. The percentages of development for the experimental group ranged from 5.9 to 8.9 (average: 7.4), and for the controls 0.9 to 3.6 (average: 1.9). Thus, the percentages for the alcoholic mice were nearly four times greater than those of the non-alcoholic controls of similar age, sex and approximate weight. The alcohol, therefore, in some way produced definite debilitation which resulted in a lowered resistance to infection.

2. *The effect on natural resistance of 45, 35, 25, and 10 per cent alcohol given to different mice for various periods of time.*—Having shown the detrimental effect

of 20 daily administrations of 45 per cent alcohol, experiments were performed to determine the effect of weaker concentrations. In the first experiment of this series, young mice two months old, on the basis used above, were divided into three experimental groups and one non-alcoholic control group. One group of experimentals was given 45, another 35, and the third 25 per cent alcohol. Table 2 shows the results of tests completed at different periods after beginning the experiment and lists for each group the average number of cysticercoids counted, the range in number of these, and the calculated percentage development.

The mice that were given 45 per cent alcohol seemed to show a reduced resistance, indicated by percentages of development ranging from 4.1 to 6.0, compared with 1.1 to 3.4 for the controls. Likewise, those given 35 per cent alcohol showed some evidence of a lowered resistance but most of them required a larger number

TABLE 1.—Comparing the percentage development of *H. nana* var. *fraterna* in alcoholic mice 2.75 months old and in non-alcoholic controls of similar age. Infecting egg dose per animal: 900

Mouse number	Sex	Number of cysticercoids	Percentage development
A. Mice given 20 daily injections of 45 per cent alcohol			
1	M	78	8.7
2	M	62	6.9
3	M	60	6.7
4	M	75	8.3
5	M	80	8.9
6	M	66	7.3
7	F	53	5.9
8	F	60	6.7
B. Non-alcoholic control mice of the same age			
1	M	20	2.2
2	M	12	1.3
3	M	10	1.1
4	M	30	3.3
5	M	15	1.7
6	M	8	0.9
7	F	32	3.6
8	F	12	1.3

of injections. With one exception, it was necessary to give 32–33 injections to obtain percentages of development (5.1–5.2) greatly different from those of controls. On the other hand, even following prolonged treatment, many mice given 25 per cent alcohol did not appear to be affected greatly.

In the second experiment, mice about two months old were divided into three experimental groups, one each on 35, 25, and 10 per cent alcohol, and one non-alcoholic control group. Test infections were given on the day after the experimentals had received 7, 14 and 26 injections respectively (Table 3).

Because of the rather small number of animals in each group, it is difficult to draw definite conclusions in this experiment. In general, however, there is some suggestion of reduced resistance in mice given 14 and in those given 26 injections of 35 per cent alcohol, and in mice given seven injections of 25 per cent alcohol. Those given 10 per cent alcohol did not appear to be affected noticeably, as the percentages of development ranged from 0.4 to 3.0, compared with 1.1 to 2.2 for the controls.

These results tend to show, in general, that 45 per cent alcohol produces a lowered resistance to infection after as few as seven injections while 35 per cent in the same volume requires longer and is less debilitating as shown by parasite numbers. Twenty-five per cent alcohol appears to be on the border line in produc-



TABLE 2.—Showing the percentage development of cysticeroids in mice given various concentrations of alcohol for different periods as compared with the development in non-alcoholic controls of similar age

Number of mice	Age in months at infection	Sex	Number of daily alcohol infections	Infecting egg dose per mouse	Nintey-three hour cysticeroids		
					Average number	Range in number	Percentage development
A. Mice given 45 per cent alcohol							
2	2.4	M	7	2000	83.5	71-96	4.2
1	2.7	M	18	1200	49.0	49	4.1
2	3.2	M	32	1000	59.5	50-69	6.0
B. Mice given 35 per cent alcohol							
2	2.4	M	7	2000	68.5	67-70	3.4
1	2.7	M	18	1200	106.0	40-106	8.8
2	3.2	M	32	1000	51.0	40-62	5.1
2	3.2	F	33	1000	51.5	40-63	5.2
C. Mice given 25 per cent alcohol							
2	2.4	M	7	2000	38.5	31-46	1.9
1	2.7	M	18	1200	115.0	115	9.6
2	3.2	M	32	1000	16.0	14-18	1.6
5	3.2	F	33	1000	38.8	30-47	3.9
D. Non-alcoholic control mice							
3	2.4	M	0	2000	22.6	20-25	1.1
3	2.7	M	0	1200	41.3	34-48	3.4
4	3.2	M	0	1000	22.7	16-30	2.3
5	3.2	F	0	1000	11.2	5-18	1.1



ing debilitation and is less consistent and effective than higher concentrations. Finally, 10 per cent alcohol given in the same volume produces little, if any, visible ill effect on resistance.

3. *The effect on resistance of 25 per cent alcohol given to some mice by mouth and to others by peritoneal route for various periods of time.*—The present experiment was designed to test whether or not alcohol given parenterally produces an effect on resistance similar to that produced by oral injections. Preliminary tests showed that because of rapid absorption following peritoneal injection, alcohol in concentration greater than 25 per cent is not tolerated well by young mice. This concentration, in the volume used above, produced intoxication rapidly but the animals recovered usually within two hours and most of them showed no visible

TABLE 3.—*Showing the percentage development of cysticercoids in mice given various concentrations of alcohol for different periods as compared with the development in non-alcoholic controls of similar age*

Number of mice	Age in months at infection	Number of daily alcohol injections	Egg dose per mouse	Average number of cysticercoids	Percentage development
A. Mice given 35 per cent alcohol					
2	2.3	7	1000	12.5	1.3
2	2.6	14	1000	37.0	3.7
2	2.9	26	1500	49.0	3.3
B. Mice given 25 per cent alcohol					
2	2.3	7	1000	40.5	4.1
2	2.6	14	1000	26.5	2.7
2	2.9	26	1500	40.0	2.7
C. Mice given 10 per cent alcohol					
2	2.3	7	1000	11.5	1.2
3	2.6	14	1000	29.7	3.0
2	2.9	26	1500	5.5	0.4
D. Non-alcoholic control mice					
6	2.3	0	1000	11.8	1.2
7	2.6	0	1000	21.9	2.2
6	2.9	0	1500	16.1	1.1

ill effects after several weeks of daily treatment. Mice about two months old were selected for the experiment and divided into experimentals and controls. One-half of the experimental animals was given 25 per cent alcohol by mouth, the others, after shaving and sterilizing the adomen and using aseptic measures, were given the same dose by peritoneal route. The first infection was given to a few animals of each group and controls ten days after beginning the experiment, and another test was made on the twenty-fifth day. This experiment was repeated with a second series of mice at which time the tests of resistance to infection were made eight and 35 days after the start of the experiment (Table 4).

In the first experiment, the animals given alcohol by mouth and those given the drug by peritoneum had about the same percentages of development (0.9 to 2.6 and 0.9 to 2.2, respectively). These percentages were very similar to those shown by the controls (0.5 to 2.6). In the second experiment, the percentages were much higher in mice of all three groups. Again those receiving the drug by mouth and by peritoneum showed about the same percentages of development (4.1 to 4.4 and

TABLE 4.—Comparing the percentage development of cysticercoids in different mice given 25 per cent alcohol by mouth and by peritoneal route with that in non-alcoholic controls of similar age

Number of mice	Age in months at infection	Number of daily alcohol injections	Egg dose per mouse	Ninety-three-hour cysticercoids		
				Average number	Range in number	Percentage development
A. Mice given 25 per cent alcohol by mouth						
4	2.2	9	1000	8.5	4-13	0.9
8	2.7	24	1000	25.7	4-60	2.6
8	2.0	7	3000	122.4	74-170	4.1
10	2.8	34	2000	87.7	48-151	4.4
B. Mice given 25 per cent alcohol by peritoneal route						
5	2.2	9	1000	9.1	7-12	0.9
8	2.7	24	1000	21.6	2-42	2.2
8	2.0	7	3000	118.9	89-163	4.0
10	2.8	34	2000	109.9	52-246	5.5
C. Non-alcoholic control mice						
4	2.2	0	1000	4.8	3-7	0.5
8	2.7	0	1000	25.6	18-40	2.6
8	2.0	0	3000	95.9	71-112	3.2
10	2.8	0	2000	43.9	15-67	2.2

4.0 to 5.5, respectively). In this case, however, the percentages were much greater than those of the controls (2.2 to 3.2). These results tend to show that the effect on resistance of 25 per cent alcohol is quite variable, being in many cases absent or hardly discernible and in others rather marked. Also, the effect of the alcohol apparently is not related to a direct action on the gastric and intestinal mucosae, since little difference in numbers of cysticercoids was noted in those receiving the drug by mouth and those given peritoneal injections.

4. *The duration of the effect of alcohol on natural resistance.*—The following experiment was planned to test whether or not lowered resistance to infection produced by alcohol persists after discontinuance of the drug. Young mice seven weeks old were matched and divided into two groups. The experimentals received by mouth the usual dose of 35 per cent alcohol which was given daily for 26 days. At this point the alcohol was discontinued so that all animals received the same number

TABLE 5.—Showing the percentage development of cysticercoids in alcoholic mice at various periods after discontinuing the drug and the development in non-alcoholic controls of similar age

Number of mice	Age in months at infection	Days after last alcohol injection	Egg dose per mouse	Ninety-three-hour cysticercoïds		
				Average number	Range in number	Percentage development
A. Mice given 26 daily injections of 35 per cent alcohol						
4	2.7	1	1000	75.0	65-81	7.5
8	2.9	8	2000	59.6	35-78	3.0
4	3.0	13	1000	19.0	10-30	1.9
3	3.9	38	1500	24.0	21-29	1.6
B. Non-alcoholic control mice of the same age						
4	2.7	0	1000	29.5	20-39	3.0
8	2.9	0	2000	69.8	50-87	3.5
4	3.0	0	1000	33.5	28-45	3.4
3	3.9	0	1500	22.0	19-26	1.5

of injections. On the following day some mice of each group were infected and a comparison made in the number of cysticeroids that developed. Later tests of resistance were made by infecting representatives of each group eight, 13, and 38 days after the alcohol injections had been discontinued (Table 5).

After the first test infection the alcoholic mice averaged 7.5 per cent development of cysticeroids as compared with 3.0 per cent for the controls. This shows that the alcohol in some way had lowered the resistance to initial infection just as in similar cases above. However, the results of the second infection given eight days after discontinuing alcohol were markedly different. At this time, the experimentals averaged 3.0 per cent development and the controls 3.5 per cent. The comparisons in percentage development of cysticeroids were about as similar as this in the last two infections given 13 and 38 days after discontinuing the alcohol. It appears, therefore, that alcoholic debilitation resulting in a lowered resistance to initial infection with this parasite is temporary only and its duration is dependent

TABLE 6.—Comparing the percentage development of cysticeroids in controls with that in mice given a single intoxicating dose of alcohol immediately before infection

Number of mice	Age in months at infection	Infecting egg dose per mouse	Ninety-three-hour cysticeroids		
			Average number	Range in number	Percentage development
A. Mice intoxicated prior to infection					
6	2.4	1500	6.7	3-10	0.4
12	2.0	900	6.1	3-12	0.7
B. Non-alcoholic control mice					
6	2.4	1500	55.0	40-69	3.7
12	2.0	900	26.7	11-48	3.0

on continued injections of the drug. At least as early as one week after alcohol is removed, natural resistance approaching normal proportions is restored.

5. *The effect on natural resistance of one intoxicating dose of alcohol given immediately before infection.*—In previous experiments, the alcoholic injections were given daily for at least one week before a test was made to determine the effect on resistance. The following experiment was performed to test the effect of one intoxicating dose given just prior to infection. Young mice about two months old were divided into two groups. Those of the experimental group were given 0.8 cc of 25 per cent alcohol by peritoneal route. After complete narcosis, they and their controls were given a test infection of *Hymenolepis* eggs (Table 6). To check on the lowering of body temperature that is known to occur during intoxication, rectal temperatures were taken every one-half hour for the first two hours after infection and on the following morning.

It will be noted that strikingly fewer cysticeroids developed in mice intoxicated just prior to infection than developed in controls. The average percentage development for the experimentals ranged from 0.4 to 0.7 compared with 3.0 to 3.7 for the controls. It is quite evident that the alcohol in some way interfered with the development of cysticeroids. Within one-half hour in most cases the temperature of the intoxicated animals fell from near 98° to 93° F. The drop was not always

as rapid but reached the level indicated or even lower within an hour and remained low for the next hour. By morning the temperature was about normal in every animal. Thus there was a definite correlation between abnormally low body temperature following infection and the development of comparatively few cysticercoids.

Another experiment was carried out to determine whether or not reduced body temperature *per se* has a demonstrable influence on the number of cysticercoids that develop. Preliminary tests showed that when mice were intoxicated and placed at once in a large 37° C incubator the body temperature returned to a near normal level usually within one-half hour, so this method was used to control body temperature after intoxication. Young mice about two months old were divided into two groups, experimental and control. The experimental animals were intoxicated with 0.8 cc of 25 per cent alcohol given intraperitoneally, and immediately they and the controls were infected. Then all of the animals were placed in the incubator overnight. Three different tests of the same kind were made (Table 7).

TABLE 7.—Showing the percentage development of cysticercoids in controls and in mice intoxicated just prior to infection then placed in a 37° C incubator to maintain normal body temperature

Number of mice	Age in months at infection	Infecting egg dose per mouse	Ninety-three-hour cysticeroids		
			Average number.	Range in number	Percentage development
A. Intoxicated mice with near normal body temperature					
5	2.5	1200	17.2	12-23	1.4
5	2.0	900	6.2	3-11	0.7
4	2.0	1000	24.0	17-31	2.4
B. Non-alcoholic control mice					
5	2.5	1200	17.8	9-27	1.5
5	2.0	900	8.2	4-12	0.9
4	2.0	1000	23.5	16-30	2.4

The results of the three tests are very similar. The experimental mice showed percentages of development that ranged from 0.7 to 2.4, whereas those of the controls ranged from 0.9 to 2.4. It was not possible to include a second control group to be kept at room temperature. However, the percentages of development for the controls used compare favorably with those recorded in experiments above from mice of this age given similar numbers of eggs. Although other factors cannot be excluded, the obvious conclusion is that one intoxicating dose of alcohol given prior to infection has no effect on the number of cysticercoids that develop provided near normal body temperature is maintained. It would appear, therefore, that reduced body temperature *per se* interferes with the development of the parasite.

#### DISCUSSION

Mice given 45 per cent alcohol daily showed a lowered resistance to *H. nana* var. *fraterna* within one week, but the most pronounced effects were seen after 20 daily injections (Tables 1 and 2). The animals on 35 per cent alcohol likewise showed reduced resistance, but in this case it usually required at least two weeks treatment (Tables 2, 3, and 5). The effect on resistance of 25 per cent alcohol was extremely variable as shown in Tables 2, 3 and 4. Even following prolonged treatment many animals appeared unaffected, while others showed some evidence of reduced resist-



ance after seven injections (Table 4). Finally, the few animals given 10 per cent alcohol for various periods of time exhibited no noticeable ill effects on resistance (Table 3). It appears, therefore, that alcohol in a concentration of 45 per cent even following a small number of doses is definitely detrimental to the natural resistance of young mice. As the concentration of the drug is decreased, it usually requires a larger number of doses to produce ill effects on this resistance. When the concentration is decreased to 10 per cent no demonstrable changes are evident. These results seem to confirm the commonly expressed view that the effect of alcohol on resistance to infection depends in large measure on the concentration consumed and the time interval of consumption.

Two observations of the above series deserve comment. One is the great differences in the susceptibility to alcohol. Individual animals show all grades of tolerance to the drug as reflected in the rapidity and duration of intoxication and its effect on physical appearance and behavior. Others have pointed out this variation in susceptibility of rabbits (Rubin, 1904; Friedenwald, 1905). Although direct tests were not carried out, there was some suggestion of a correlation between tolerance to the drug and the degree of debilitation produced. The other observation was the percentage development of cysticercoids in the control mice. It will be noted from the various tables that the percentages are considerably lower than in previous studies in which the eggs for infections were stored before isolation (Larsh, 1943b). The use of unstored eggs in the present study probably accounts for the lower rates, since these percentages compare fairly well with those obtained elsewhere under similar experimental conditions (Larsh, *ibid.*).

As mentioned above, certain factors have been shown to have an influence on the natural resistance of mice to infection with *H. nana* var. *fraterna*. Age alone seems to be influential as mice less than one month or over four months are strikingly more resistant to infection than those about two and one-half months old (Shorb, 1933; Hunninen, 1935; Larsh, 1943a). Increased resistance to initial infection is also shown by mice infected simultaneously with *Nippostrongylus* (Larsh and Donaldson, 1944). On the other hand, under certain experimental conditions mice may exhibit decreased resistance to infection. This is shown by old mice splenectomized when young. These were much more susceptible to an initial infection than mice of the same age with intact spleens (Larsh, 1944). Also, as demonstrated above, alcohol in high concentrations produces a high grade debility shown by increased susceptibility to infection. Just how these stimulating or debilitating effects are produced is as yet unknown, but the mechanism should be studied further because of its importance in explaining the function of natural resistance.

#### SUMMARY

The effect of alcohol on the natural resistance to *H. nana* var. *fraterna* has been studied in a large number of white mice. In general, 45 per cent alcohol by mouth produced definite debilitation after 20 daily injections and some impairment of resistance within one week, whereas 35 per cent, although producing debilitation in many cases, required a longer period of time. A concentration of 25 per cent alcohol produced extremely variable effects on resistance while 10 per cent did not appear to produce change. These results seem to confirm the commonly expressed



opinion that the effect of alcohol on resistance depends largely on its concentration. The effect of the drug differed very little when given by mouth and by peritoneum which showed that possible gastric and intestinal damage following oral administration has little, if any, influence on the number of parasites that develop. Lowered natural resistance produced by the drug was temporary and disappeared in all cases within one week after discontinuing treatment. Finally, one intoxicating dose of alcohol prevented the development of usual numbers of cysticercoids from a test infection given soon after narcosis. If normal body temperature was maintained by artificial means, however, similar numbers developed in the intoxicated mice and their non-alcoholic controls. This is interpreted to mean that abnormally low body temperature, evident during intoxication, interferes with the development of the parasite.

## REFERENCES

- DOYEN, E. 1885 Recherches anatomiques et expérimentales sur le choléra épidémique. Arch. de Physiol. Norm. et Path. 6: 179-236.
- FRIEDENWALD, J. 1905 The pathologic effects of alcohol on rabbits. J. A. M. A. 45: 780-784.
- GOLDBERG, S. J. 1901 Ueber die Einwirkung des Alkohols auf die natürliche Immunität von Tauben gegen Milzbrand und auf den Verlauf der Milzbrandinfektion. Centralbl. f. Bakt. I Abt. 30: 696-700; 731-741.
- HUNNINEN, A. V. 1935 Studies on the life history and host-parasite relations of *Hymenolepis nana* (*H. nana* var. *fraterna* Stiles) in white mice. Am. J. Hyg. 22: 414-443.
- KOCH, R. 1885 Konferenz zur Erörterung der Cholerafrage (Zweites Jahr). Deutsche Med. Wchnschr. No. 37A, 12 September, 1-60 pp.
- KRUSCHLIN, A. W. 1909 Ueber die Wirkung des Alkohols auf die Tätigkeit der Phagocyten. Ztschr. f. Immunitätsforsch. u. Exper. Therap. 1: 407-421.
- LAITINEN, T. 1900 Ueber den Einfluss des Alkohols auf die Empfindlichkeit des Thierischen Körpers für Infektionsstoffe. Ztschr. f. Hyg. u. Infektionskr. 34: 206-252.
- LARSH, J. E., JR. 1943a The relationship between the intestinal size of young mice and their susceptibility to infection with the cestode, *Hymenolepis nana* var. *fraterna*. J. Parasitol. 29: 61-64.
- 1943b Increased infectivity of the eggs of the dwarf tapeworm (*Hymenolepis nana* var. *fraterna*) following storage in host feces. J. Parasitol. 29: 417-418.
- 1944 The relation between splenectomy and the resistance of old mice to infection with *Hymenolepis nana* var. *fraterna*. Am. J. Hyg. 39: 133-137.
- LARSH, J. E., JR. AND DONALDSON, A. W. 1944 The effect of concurrent infection with *Nippostrongylus* on the development of *Hymenolepis* in mice. J. Parasitol. 30: 18-20.
- PARKINSON, P. R. 1909 The relation of alcohol to immunity. Lancet 2: 1580-1582.
- PICKRELL, K. L. 1938 The effect of alcoholic intoxication and ether anesthesia on resistance to pneumococcal infection. Bull. Johns Hopkins Hosp. 63: 238-260.
- RUBIN, G. 1904 The influence of alcohol, ether, and chloroform on natural immunity in its relation to leucocytosis and phagocytosis. J. Infect. Dis. 1: 425-444.
- SHORE, D. A. 1933 Host-parasite relations of *Hymenolepis nana* var. *fraterna* in the rat and the mouse. Am. J. Hyg. 18: 74-113.
- THOMAS, 1893 Ueber die Erzeugung der Cholera von der Blutbahn aus die prädisponierende Rolle des Alkohols. Arch. f. Exper. Path. u. Pharmakol. 32: 38-48.

## THE MORPHOLOGY OF *TAMERLANIA BRAGAI* DOS SANTOS, 1934

HORACE W. STUNKARD

New York University, University Heights, N. Y.

*Tamerlania bragai* was described by Dos Santos (1934) as *Tamerlanea bragai* (obvious misspelling of *Tamerlania*) from the kidneys and excretory ducts of domestic pigeons and chickens at Rio de Janeiro. It was reported from the pigeon in São Paulo by Reis and Nobrega (1936), in the Philippines by Tubangui and Masiluñgen (1941), in Puerto Rico by Maldonado and Hoffman (1941) and from turkeys in Brazil by Barretto and Filho (1942). All of the Latin American authors spelled the generic name "*Tamerlanea*" and none of the accounts reported the presence of an acetabulum.

The genus *Tamerlania* was erected by Skrjabin (1924) to contain *T. zarudnyi* from *Passer montanus* in Russian Turkestan. Khitrowo-Kalantarian (1925), from original observations and from information communicated by Skrjabin and by Issaitschikow, reported the parasite from four additional species of birds and stated that the digestive ceca unite posteriorly. Other described species include *T. meruli* by Nezlobinski (1926), *T. bragai* by Dos Santos (1934), *T. japonica* by Yamaguti (1935) and *T. melospisae* by Penner (1939). Yamaguti (1941) reported that *T. japonica* is specifically identical with *T. zarudnyi*. Penner formulated a key to the species of the genus, the original diagnosis of which was emended or amplified by each of the subsequent writers. Whether an acetabulum is present in species other than *T. bragai* remains to be determined.

Skrjabin (1924) also erected the family EUCOTYLIDAE to contain *Eucotyle* Cohn, 1904 and two new genera, *Tanaisia* and *Tamerlania*. Two additional genera, *Lepidopteria* and *Ohridia* were added by Nezlobinski (1926). The family characters were stated by Fuhrmann (1928) and reviewed by Cheatum (1938) and Penner (1939). According to these authors the worms are monostomes, parasites of the renal ducts of birds. The family is world-wide in distribution, the hosts are chiefly migrants, and several of the species have been described on the basis of limited material. As a result, the morphology is imperfectly known and the validity of certain genera and species is questionable.

In a personal communication, dated September 25, 1942, Dr. William A. Hoffman wrote that one of his students, Mr. José F. Maldonado, had found that *T. bragai* possesses a small ventral sucker. Dr. Hoffman stated that after publication of the note, Maldonado and Hoffman (1941), Mr. Maldonado had discontinued his studies on *T. bragai*, but that the presence of an acetabulum in the species should be of interest in determining the phylogeny of the monostomes and offered, with the reservation that the discovery of the acetabulum should be accredited to Maldonado, to place the material at my disposal. The material sent by Dr. Hoffman consisted of one mounted specimen (Fig. 1) and an incomplete set of serial sections of the kidney. The study was completed and a manuscript prepared, giving credit to Mr. Maldonado for discovery of the acetabulum. Subsequently, Mr. Maldonado returned to Puerto Rico and continued his studies on *T. bragai*. Fol-

Received for publication, May 8, 1945.

lowing correspondence and a conference with me, Maldonado (1943) published a preliminary note announcing the discovery of the acetabulum and of the life cycle, and suggested the publication of two separate papers, preferably simultaneously and in the same journal, his to deal with the life history, epidemiology and pathology of *T. bragai* and mine to consider the morphology and taxonomy of the parasite. Accordingly, my account was withheld and his, received for publication May 8, 1945, appears in this issue of the Journal of Parasitology.

The specimens from Puerto Rico do not agree completely with the original description of *T. bragai*, and to check their specific identity, Professor Travassos sent additional material from the Instituto Oswaldo Cruz. Grateful acknowledgment is made her for his kindness and cooperation. The specimens from Rio de Janeiro, sent by Travassos, are morphologically and presumably specifically identical with those from Puerto Rico. Although they do not agree in all particulars with the description of *T. bragai* given by Dos Santos (1934), there are strong reasons for believing that they belong to the same species. Differences between these specimens and Dos Santos' account are noted in the following description.

*Tamerlania bragai* Dos Santos, 1934

The body is flattened dorsoventrally, with almost parallel sides and rounded ends. Both small, sexually immature, and large, gravid worms may be present together in long-established infections. This observation and the presence of hundreds of specimens in a single kidney, indicate repeated infection with the development of little or no immunity. A specimen 0.6 mm long is immature; gravid worms measure 1.2–3 mm long, 0.2–0.45 mm wide. In cross sections the body is oval; the thickness varies inversely as the width. In large tubules the worm is usually flatter; in small tubules it may be almost round in section. An acetabulum (Figs. 1, 4) is regularly present, 0.04–0.05 mm in diameter, situated in the anterior part of the middle third of the body. The thickness of its wall is about equal to the diameter of the lumen and the organ is probably functionless. The body wall of the worm consists of circular, longitudinal and oblique muscle sheets, but all are weak and poorly developed. The parenchyma is loosely organized and, in fully mature specimens, most of the body is occupied by the gravid coils of the uterus. The cuticula bears flat, chisel-like, recurved spines (Figs. 2, 3). The base is a flattened oval, thicker and often slightly wider than the blade which tapers to a thin edge. The largest ones measure about 0.008 mm wide at the base and about 0.01 mm long. Frequently the blade may be resolved into several (3–8) fused elements with as many distinct points at the free edge.

The mouth is subterminal; the oral sucker is well developed, 0.1–0.145 mm in diameter. (Dos Santos gave the size as 0.5 mm wide and 0.41 mm long, but his figure shows the sucker about one-half the body width which he stated was maximally 0.66 mm.) The oral sucker communicates directly with the pharynx, which usually indents the posterior wall of the oral sucker, and which measures 0.045–0.065 mm in diameter. (Dos Santos measurement is 0.16 mm.) The pharynx is followed by a short esophagus, recognizable only when the anterior end of the worm is extended. In sections, the epithelium of the digestive ceca extends to the pharynx. The ceca, of variable diameter, unite near the posterior end of the body (Fig. 1), thus forming a loop, the ends of which are almost equidistant from the corresponding ends of the body. The ceca contain a granular coagulum; no nuclei of ingested cells were observed, but Fig. 5 suggests that the worms may attack the renal epithelium.

The excretory pore is terminal and the vesicle could be traced forward, ventral to the uterine coils, as far as the level of the intestine.

The testes are opposite or slightly diagonal in position and frequently overlap each other in the median plane. They are situated a short distance behind the ovary, in the posterior part of the anterior half of the body, and at the level of the acetabulum. When small, they are dorsal in position. They are oval, longer than wide, slightly lobed, and difficult to measure. In mature specimens they are 0.12 by 0.09 mm to 0.22 by 0.18 mm. (According to Dos Santos the testes are 0.5 mm long and 0.35–0.47 mm wide, but in his figure they are represented as smaller than the ovary for which he gave the measurement 0.15 mm long by 0.22 mm wide.) Vasa efferentia arise at the anteromedian faces of the testes, pass forward and mediad, dorsal to the ovary. They enter separately into the large, posterior end of a pyriform seminal vesicle,

which is closely invested by a muscular wall and may be regarded as a cirrus sac. The vesicle is median, lies in part dorsal and in part anterior to the ovary, and measures 0.06–0.07 mm in diameter. Ventrally the vesicle is continuous with a short, muscular canal which leads to the shallow genital sinus. There is a space, occupied by loose mesenchymatous tissue, between the canal and the muscular wall of the sac. Dos Santos described the structure as “un tout petit cirre atrophé.” The genital pore is median, ventral, below the anterior end of the ovary.

The ovary is lobed, slightly wider than long, and measures 0.1–0.2 mm in diameter. (According to Dos Santos the ovary is 0.15 mm long and 0.22 mm wide.) It is situated about one-third of the body length from the anterior end; it occupies the region between the ceca but is slightly displaced toward one side, left or right, by the uterus which passes forward on the opposite side. The oviduct arises at the dorsal surface, bends dorsad, mediad and backward where it receives a very short duct from a small seminal receptacle. No Laurer's canal was observed, but fixation of the tissue was not good enough to preserve delicate structures perfectly. The oviduct then receives a short duct from the vitelline receptacle and passes ventrad through Mehlis' gland to form the initial part of the uterus. The uterus passes backward, dorsal to or between the testes (Fig. 4) and turns toward the ovarian side; it continues in loops and coils almost to the posterior end of the body where it turns forward and returns in the same manner, passes between and below the testes (Fig. 4), makes a transverse loop between the testes and the ovary, and passes forward on the antovarian side of the body above the digestive cecum but median to the vitelline follicles at that level. It continues in irregular coils to the region of the pharynx and back to the level of the ovary where a short, thick-walled metraterm leads to the genital sinus. The vitellaria consist of well-developed follicles which form almost continuous columns in the extracecal areas. They extend forward about one-half the distance from the genital pore to the anterior end of the body and backward about one-half of the distance from the ovary to the posterior end of the body. (Dos Santos stated, “Les vitellogènes sont placés exclusivement aux champs latéraux, pré et retrotesticulaires, depuis 0.32 mm de l'extrémité antérieure de la ventouse orale jusqu' à 0.32 mm de l'extrémité postérieure du trematode.” In his figure, however, the vitellaria have the same distribution as shown in Fig. 1, and are at least twice as far from the posterior as from the anterior end of the body.) Vitelline ducts pass mediad dorsal to the ceca, at the level of the ootype and unite to form the vitelline receptacle. Eggs in the initial portion of the uterus have thin, transparent shells and contain ova; those in the terminal portion have thick, yellow shells and contain fully formed miracidia. The eggs are operculate, often slightly flattened on one side, and measure 0.034 by 0.015 mm. (According to Dos Santos, the eggs measure 0.031 by 0.013 mm; although I have measured eggs of this size, in the present material the average is slightly larger.)

*Tamerlania bragai* is the only member of the EUCOTYLIDAE whose life history is known and the only one in which an acetabulum has been reported. According to Maldonado, the sucker appears early in the development of the cercaria and fails to continue its growth, thus suggesting that here it is a vestigial rather than a rudimentary structure. The discovery of an acetabulum in this species affords new and significant information touching on the status of the suborder, MONOSTOMATA. The phylogeny of the monostomes was discussed by Stunkard (1934) and a further consideration of the subject is to appear in a forthcoming number of Biological Reviews of the Cambridge Philosophical Society.

#### LITERATURE CITED

- BARRETTO, J. F. AND MIES FILHO, A. 1942 Primeiras observações sobre a presença de “*Tamerlanea bragai*” (Violantino Santos, 1934) nos rins de *Meleagris gallopavo domestica*. Ministerio Agric. Inst. Biol. Animal, Rio de Janeiro, Brazil: 3 pp.
- CHEATUM, E. L. 1938 *Tanaisia pelidnae* n. sp. and *Orchipedium tracheicola* (Trematoda). J. Parasitol. 24: 135–141.
- FUHRMANN, O. 1928 Vermes Amera: Trematoda, in Kükenthal-Krumbach, Handbuch der Zoologie 2: 1–128.
- KHITROWO-KALANTARIAN, E. 1925 Zur Diagnose der Trematodengattung *Tamerlania* Skrjabin. Centr. Bakt., II Abt. 63: 255–256.
- MALDONADO, JOSÉ F. 1943 A note on the life cycle of *Tamerlanea bragai* Santos, 1934 (Trematoda: Eucotylidae). J. Parasitol. 29: 424.
- 1945 The life cycle of *Tamerlania bragai* Santos, 1934 (Eucotylidae), a kidney fluke of domestic pigeons. J. Parasitol. 31: 306–314.



- MALDONADO, JOSÉ F. AND HOFFMAN, W. A. 1941 *Tamerlanea bragai*, a parasite of pigeons in Puerto Rico. J. Parasitol. 27: 91.
- NEZLOBINSKI, N. 1926 Helmintološke studije u Ohridskoj Kotlini. I. O. bubrežnim Trematodama kod ptica. Glasnik Tsentral. khig. Zavoda, Beograd, 1: 202-217.
- PENNER, LAWRENCE R. 1939 *Tamerlania melospizae* n. sp. (Trematoda: Eucotylidae) with notes on the genus. J. Parasitol. 25: 421-424.
- REIS, J. AND NOBREGA, P. 1936 Tratado de doenças das Aves. Trabalho Inst. Biol. São Paulo; pp. 1-469.
- SANTOS, VIOLANTINO DOS 1934 Monostomose renal das aves domesticas. Rev. Dept. Nac. Prod. Animal, Brazil, 1: 203-215.
- SKRJABIN, K. J. 1924 Nierentrematoden der Vögel Russlands. Centr. Bakt., II Abt. 62: 80-90.
- STUNKARD, HORACE W. 1934 The life history of *Typhlocoelum cymbium* (Diesing, 1850) Kossack, 1911 (Trematoda, Cyclocoelidae); a contribution to the phylogeny of the monostomes. Bull. Soc. Zool. France 59: 447-466.
- TUBANGUI, MACROS A. AND MASILUNGEN, V. A. 1941 Trematode parasites of Philippine vertebrates, IX: Flukes from the domestic fowl and other birds. Philip. J. Sci. 75: 131-141.
- YAMAGUTI, S. 1935 Studies on the helminth fauna of Japan. Part 5. Trematodes of birds, III. Jap. J. Zool. 6: 159-182.
- 1941 Studies on the helminth fauna of Japan. Part 32. Trematodes of birds, V. Jap. J. Zool. 9: 321-341.

## EXPLANATION OF FIGURES

ac—acetabulum	ph—pharynx
ev—excretory vesicle	sv—seminal vesicle
in—intestine	ts—testis
os—oral sucker	vt—vitellaria
ov—ovary	

FIG. 1. Whole mount, ventral view, specimen from Puerto Rico. It measures 1.31 mm long, 0.252 mm wide and probably is not completely mature.

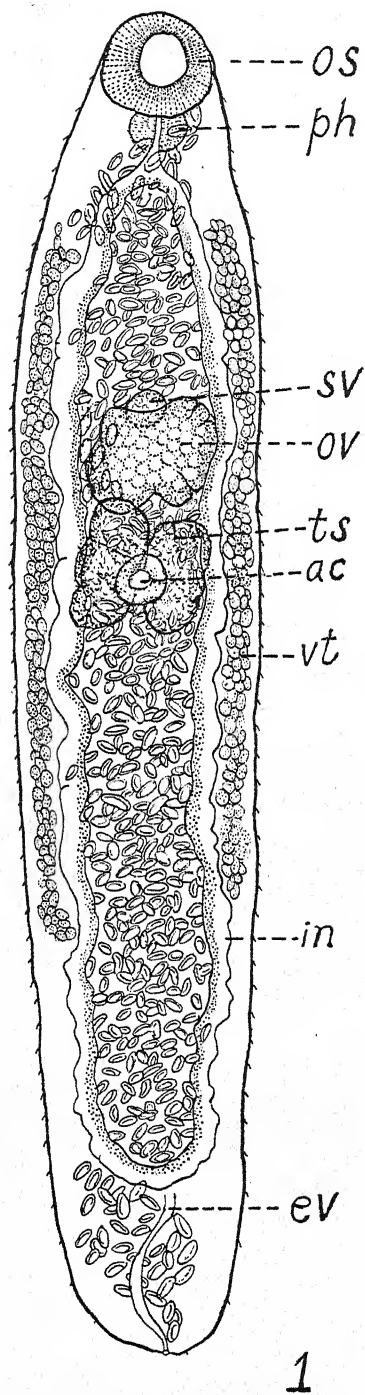
FIG. 2. Normal spines; 2a side view, 2b front view.

FIG. 3. Variations observed in spines.

FIG. 4. Cross section of mature specimen at the level of the acetabulum, showing testes, vitellaria, intestinal ceca, the descending limb of the uterus with thin-shelled eggs on the dorsal and the ascending limb with thick-shelled eggs on the ventral side of the body. Specimen from Rio de Janeiro, 0.3 mm wide.

FIG. 5. Sagittal section of the anterior end of a worm, situated where a small tubule opens into a large one, showing a portion of the excretory tubule drawn into the oral sucker; the epithelium is absent and the underlying tissues distorted.





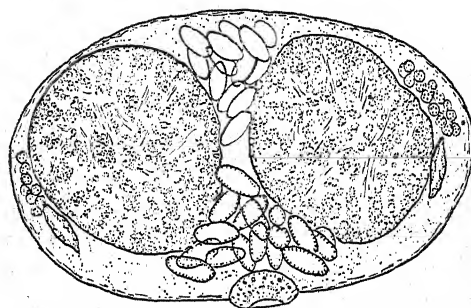
2a



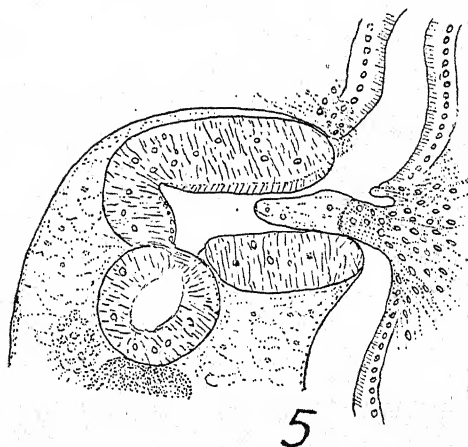
2b



3



4



5

# THE LIFE CYCLE OF *TAMERLANIA BRAGAI*, SANTOS 1934, (EUCOTYLIDAE), A KIDNEY FLUKE OF DOMESTIC PIGEONS

JOSÉ F. MALDONADO

Department of Medical Zoology, School of Tropical Medicine, San Juan, P. R.

In the course of study of several Puerto Rican trematodes the author obtained a larval form from specimens of the terrestrial snail *Subulina octona*, collected in the neighborhood of a pigeon house. In a preliminary study, Maldonado (1943) identified this form as *Tamerlania bragai*, a trematode inhabiting the urinary passages of domestic pigeons in Brazil and Puerto Rico. The present report describes in detail the life cycle of this parasite.

## MATERIALS AND METHODS

The intramolluscan phase of the cycle was studied by experimental infection of laboratory-raised *Subulina octona*. Previous trials had demonstrated that eggs of *Tamerlania* fail to hatch in the open. The snails were therefore exposed by allowing them to feed on sedimented droppings of parasitized pigeons. Every four days after the period of exposure, two snails were removed from their shells and fixed in Zenker's solution. They were sectioned serially and stained in Bullard's hematoxylin-eosin, although the more advanced stages of development were studied *in vivo* preferably. The miracidium was studied by removing the gut of snails within one-half hour after they were observed ingesting the excreta and examining directly under the microscope or in sections.

The process of infection and development of the fluke in the pigeon was determined by feeding infected snails to young birds and killing subsequently at various intervals beginning six hours after infection. The parasites were fixed in corrosive sublimate-acetic acid and stained in Ehrlich's acid hematoxylin or in Alum cochineal-Delafield hematoxylin.

## EXPERIMENTAL RESULTS AND OBSERVATIONS

*The egg* (Fig. 1).—The egg of *Tamerlania bragai* has a thick, brown, smooth shell which is elongated and slightly asymmetrical. It is provided with an operculum anteriorly and with a small protuberance posteriorly. The egg measures on the average 30 microns in length by 15 microns in width. The miracidium is fully developed and infective at oviposition. It fills practically all the egg cavity except for a narrow space at the anterior end occupied by refractile granules. All unhatched miracidia present a triangular, fibrillous mass slightly anterior to the middle of the body.

Eggs are usually observed in great numbers in the fluid part of the droppings. In heavy infections the irritative action of the parasite causes an increase in excretion so that urine practically free from feces is often passed. In these cases, eggs are obtained in almost pure concentrations. The examination of solid excrement, however, constitutes a reliable means of diagnosis due to the admixture of feces with urine in the cloaca. Under favorable conditions of humidity and aeration the

Received for publication, May 8, 1945.

egg may remain infective for a considerable time. In well-aerated charcoal cultures or in clean rain water it lives for over a month.

*The miracidium* (Fig. 2).—Hatching takes place in the distal end of the stomach of the mollusc. The process is identical with that observed in other trematodes. Within fifteen minutes after the egg arrives in the crop the operculum is suddenly released and the embryo is extruded within its embryophore. Tearing out from this envelop is relatively easy and the miracidium is then free to move in the lumen of the snail gut.

The minute size and cryptic habits of the miracidium did not permit its study in detail. No miracidia could be freed from the egg by artificial means. It was thus necessary to study them through the gut wall, a circumstance which contributed to obscure the details. Fig. 2 is therefore a diagrammatic representation of the miracidium as observed within the intestine of the snail. The larva is about 30 microns long, pyriform, with a small anterior snout-like projection. Ciliation is uniform, giving the organism a smooth motion which is accompanied by peristaltic contractions. The triangular, fibrillous mass of the unhatched form could not be observed. The parenchyma possesses scattered refractile granules. In sections, a pair of delicate nuclei representing the germ cells, were observed.

*The mother sporocyst*.—The miracidium may reach the tissues of the snail by piercing the intestinal epithelium. This route of migration is suggested by the presence of early developmental stages in the peri-intestinal connective tissue, in the kidney and in adjacent parts of the digestive gland.

Within four days after ingestion of the egg, the miracidium has transformed into a mother sporocyst which appears as a round, solid mass of cells with deeply staining nuclei. On the eighth day it has grown diffusely among the tissues and only loosely scattered groups of germ cells lacking definite organization can be observed. On rare occasions a thin membrane may be seen enclosing these cells. The parasite has apparently relinquished, to an extreme degree, its somatic capabilities for the germinative ones. Beyond the eighth day the increase in number and size of the germ balls obscures the outlines of the mother sporocyst. The production of new germ balls continues for a considerable time. However, the germinative capacity depends to a great extent on space relations within the host. The duration of the mother sporocyst has been established as two months. Beyond this time the size of the brood mass prevents further proliferation and all the daughter sporocysts eventually reach the same stage of development.

*The daughter sporocyst* (Figs. 3 and 4).—The development of the second generation of sporocysts resembles closely that of most trematodes. The germ cells in the mother form may divide and separate repeatedly, but when some fail to do so a germ mass arises. In *Tamerlania bragai* this process takes place as early as the fourth day of life in the snail, at the time when the mother sporocyst is still a solid structure. At this stage a number of cells with different staining properties may be observed forming a compact group at the center.

The germ balls grow by cell multiplication. Eight to ten days after arising, they break out of the mother sporocyst. The majority remain about the middle of the snail body, forming a compact mass which distorts the digestive gland considerably. Others may move to more distant parts.

Around the twelfth day, differentiation of the germ mass into the ultimate form begins. A body cavity develops and a cercarial germ mass may segregate simultaneously from the body wall. At maturity the daughter sporocyst becomes a passive, simple, spherical or ovoid, saccular structure enclosing a few cercariae at various stages of development. The body wall is very thin and undifferentiated. The size varies with the number of cercariae. Those in which a single cercaria has arisen measure about 200 microns in diameter, others with eight or ten cercariae vary between 500 and 600 microns in diameter.

*The cercaria* (Fig. 5).—The cercariae of *Tamerlania bragai* arise, as in other trematodes, by the proliferation of the germ cells within the daughter sporocyst. Although a sporocyst may give rise, simultaneously with the formation of the body cavity, to only one individual, the majority of the sporocysts produce several cercariae at different intervals during development. The latter may be seen to arise from germ cells lying along the body wall of the sporocyst. These form small masses which project for a time into the cavity and later detach. Following a period of growth by cell multiplication the germ ball elongates.

The suckers are the first organs to appear. An acetabulum arises as a knob near the midbody. The oral sucker develops by a constriction of the most anterior part of the body. The buccal cavity first appears as an anterior depression in the oral sucker. Because of greater growth of the dorsum this sucker and its cavity will come to lie subventrally as development proceeds. The pharynx in its earliest stage is prominent, about half as large as the oral sucker. An excretory vesicle arises by fusion of the distal parts of two lateral collecting tubules in the elongating germ mass. The secretory system is formed simultaneously with the other organs. Cystogenous glands arise from cells scattered throughout the body, since numerous aggregations of greenish secretory granules may be observed in early stages. There is no evidence of the formation of ducts.

The first fully developed cercariae appear on the fourth week of infection. The organism is markedly delicate. It is entirely unable to survive outside of the host or even to leave the shelter of the sporocyst. Its activities are restricted to slow movements of the body, particularly of the fore part which is most often curved ventrad (Fig. 5) thus causing the parasite to lie on its side. The body is cylindrical, tapering slightly posteriorly and covered with a thin, rugose cuticle. The pre-acetabular region is thicker because of the greater size of those cystogenous glands which lie in it.

The oral sucker, which lies subventrally, is an elongated, muscular structure. The oral cavity is funnel-like and divides the sucker into a small, lower lip and a large, upper one. The acetabulum is globular and stands out prominently, the sucker cavity varying in size and shape with the state of contraction. The clavi-form excretory bladder is the only observable portion of the excretory system. The flame cells and ducts are obscured by the cystogenous glands. A rudimentary tail is represented by a small stub in some cercariae. In others the posterior tip of the body is twisted, giving a false impression of a tail rudiment.

Average body measurements of living cercariae are: Length of body, 400 microns; width at middle, 50 microns; oral sucker—length, 45 microns; acetabulum—diameter, 25 microns; pharynx—diameter, 18 microns; oral sucker to acetabulum, 150 microns; acetabulum to tip of body, 210 microns.



The *metacercaria* (Figs. 6 and 7). As early as the thirtieth day of infection those cercariae which have attained maturity proceed to encyst within the sporocyst. The actual process of encystation was not observed. It is supposed that the glandular secretions are extruded through the integument since no ducts could be outlined. The cyst formed is leathery, elastic and transparent, between 4.0 and 7.5 microns in thickness. The entire structure is oval, measuring about 146 microns by 36 microns. In old infections the encysted cercariae may be found in masses still surrounded by remnants of the disintegrating sporocyst, although in many cases the latter have entirely disappeared.

Encystment marks a turning point in the organization and behavior of the parasite. From then on it may be seen to move vigorously and almost constantly within its enclosure. During this stage the larva becomes prepared to endure the arduous journey to its final habitat in the definitive host.

When freed from the cyst by the normal process of digestion in the pigeon, the metacercaria appears as a flat, leaf-like and delicate organism, measuring about 360 microns in length. This shape is evidently attained upon the discharge of the cystogenous secretions. The body covering has become thick and striated, with short, blunt papillae which are more numerous on the anterior margin of the oral sucker.

The oral sucker is subventrad, perfectly round when seen *en face* and about 36 microns in diameter. The oral cavity is large and leads into a muscular pharynx about 18 microns in diameter. A very short esophagus opens from the pharynx and bifurcates into the thin, undulating crura which unite posteriorly. The acetabulum is globular and muscular, almost as large as the oral sucker, and protrudes markedly from the ventral surface. When expanded, the acetabular cavity is shallow and hemispherical.

The excretory system is clearly visible. The bladder is thin, undulating, and opens ventrad. It extends to the level of the acetabulum where it bifurcates at right angles into two main trunks each of which in turn subdivides into an anterior and a posterior secondary duct. The former is convoluted and extends forward turning back on itself at the level of the oral sucker to end in the vicinity of the acetabulum. The latter is undulated and ends at the posterior end of the body. The flame cells are forty in number, arranged in pairs on the sides of the body. Their relative position varies but in general eight pairs are located preacetabulad and the other twelve postacetabulad.

A small compact mass of cells in front of the acetabulum constitutes the genital primordium. Two sets of small glands, about ten in total, lie behind the bifurcation of the esophagus. The ducts from each set project forward over the dorsal surface of the oral sucker, forming a complex. There is no evidence of an external opening. These glands probably correspond to the cephalic glands of other trematodes and may already be present in the cercaria although obscured by the cystogenous glands.

#### *Development in the Final Host*

*Mode of infection of the pigeon.*—Pigeons acquire the infection only upon the ingestion of the infective snail. Birds are markedly fond of snails which they supposedly use as grit. In the case of *Subulina octona*, the mollusc under consideration, the shiny, cream-colored shell acts as an added attraction. The facility with



which the parasite is acquired in nature is expressed in the high incidence of infection, as much as 90 per cent in many localities. Parent birds may even infect their brood by feeding them infective material.

Ingested snails are crushed in the gizzard and the liberated metacercariae pass into the duodenum where they readily excyst. Five to six hours after feeding infective snails the actively moving metacercariae may be obtained from the entire length of the digestive tract, in the cloaca and along the ureters. Some may even have reached the kidneys by the latter route. In every instance this has been the only proved mode of arrival in the kidneys.

*Development of the adult.*—On the fourth day of life in its final habitat (Fig. 8) the parasite measures about 600 microns in length, that is, over twice its original size. The shape of the body and arrangement of the organs are practically the same as in the metacercaria. The most marked structural alterations are the enlargement of the genital primordium and the segregation of the ovary from its anterior portion as a round cell mass.

On the eighth day (Fig. 9) the remaining portion of the genital anlage has differentiated into a pair of testes which lie close together immediately behind the ovary, two small masses representing the primordia of the vitellaria, located directly over the gut on each side of the acetabulum, and a strand of cells extending back dorsad to the acetabulum representing the beginnings of the uterus. At this stage the parasite has attained a length of about 950 microns. The body covering is considerably changed due to the deepening striations which have begun to acquire the shape of spines. The oral sucker, the pharynx and the intestine have increased correspondingly in size. The acetabulum, although showing slight enlargement, has failed to grow at the same rate.

Between the eleventh and the fifteenth day (Fig. 10) the fluke attains adulthood. The first eggs lie already in the distal end of the uterus near the genital opening, but the majority appear only as distorted, empty shells. The vitellaria, testes and ovary are fully developed and functional. The relative position of the testes has changed. The post-acetabular body region has markedly elongated to make room for the enlarging uterus. A small yolk sac is located just in front of the testes. The seminal vesicle is large, extending over the dorsal surface of the ovary. At this stage the worm is about 1.25 mm long. The body covering is definitely spiny. The acetabulum is atrophic, measuring less than 40 microns in diameter, or even absent in some specimens.

From now on the morphological changes consist of enlargement of the whole body and gradual accumulation of eggs until they obscure practically all the organs. Oviposition starts during the fourth week of infection.

#### DISCUSSION

The present observations on the life history of *Tamerlania bragai* are of interest not only because they constitute the first record on the life history of members of the family EUCOTYLIDAE; but also because they shed some light on the phylogensis of this group of parasites and thus help to clarify their true systematic position.

The family EUCOTYLIDAE was created by Skrjabin (1924) for digenetic monostomes of the urinary organs of birds. It includes five genera: *Eucotyle* Cohn 1904, *Tanaisia* Skrjabin 1924, *Tamerlania* Skrjabin 1924, *Ohridia* Nezhlobinski

1926 and *Lepidopteria* Nezlobinski 1926. The genus *Tamerlania* was originally created for eucotylids without the anterior muscular prominence typical of *Eucotyle*, with vitellaria extending from the testes backwards, with testes located close together, side by side and with esophagus lacking. It was later modified by several authors (Penner, 1939) to include the presence of a short esophagus, the union of the intestinal crura at the posterior end in some species and the extension of the vitellaria in front and back of the testes. On this basis the genus includes four species: *T. zarudnyi* Skrjabin 1924, *T. meruli* Nezlobinski 1926, *T. bragai* Santos 1934 and *T. melospizae* Penner 1939. A fifth form, *T. japonica* Yamaguti 1935, was considered at a later date synonymous with *T. zarudnyi* by its creator (Yamaguti, 1941).

The taxonomy of these trematodes, both in the genus *Tamerlania* and in the family EUCOTYLIDAE, has been based entirely on adult characters, host relations and geographical distribution. Since nothing was known of the particular life cycles, the larval characters could not be used in their classification. As far as *Tamerlania bragai* is concerned, the presence of an acetabulum during larval development as demonstrated in this work makes its present taxonomic position in the suborder MONOSTOMATA untenable. This example is a further addition to the already long list of cases in the literature which show that the classification of trematodes should not be based exclusively on the characteristics, morphological or otherwise, of the adult form. The significance of these observations in respect to the other members of the family will be determined only upon the elucidation of their respective life cycles.

The acetabulum of *Tamerlania bragai* was first observed by the author five years ago in a young adult specimen obtained from a naturally infected pigeon. The organ was subsequently demonstrated in other specimens upon sectioning. This material was forwarded to Dr. Horace W. Stunkard of New York University for a detailed study. The result of his observations will appear in a separate article.

#### SUMMARY AND CONCLUSIONS

The life history of *Tamerlania bragai*, a fluke of the urinary passages of pigeons, described originally by Santos (1934) from Brazil and later reported from Puerto Rico by the present author, is described. This is the first record on the life history of members of the family EUCOTYLIDAE.

*Subulina octona*, a land snail, acts as intermediate host. The egg is ingested by the snail and hatches in the crop. The miracidium invades the connective tissue and develops there into a very delicate mother sporocyst. This gives rise to numerous daughter sporocysts, each of which in turn gives rise to a few cercariae. The cercaria is a highly specialized organism which spends its entire life in the sporocyst and eventually encysts within it. The larval cycle is completed in a month. Pigeons acquire the infection upon the ingestion of snails bearing encysted cercariae. After excystation in the duodenum the metacercariae move down into the cloaca from where they pass into the ureters and thence finally to the kidneys. Some parasites may reach these organs as early as five hours after the snail is ingested by the pigeon. The fluke becomes sexually mature at the end of the second week. Eggs may be recovered in the urine and excreta of the pigeon on the twenty-third day.

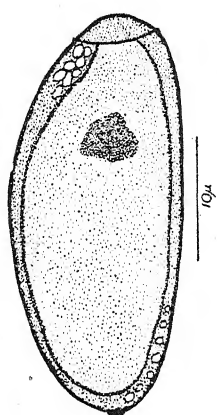
The presence of an acetabulum in this parasite demands a revision of its taxonomy. This organ occurs in all the cercariae and young adults. In the latter it may grow slightly, but eventually atrophies and as a rule cannot be observed at sexual maturity. A reconsideration of the taxonomy of the other members in the group must await further knowledge of their biology.

## BIBLIOGRAPHY

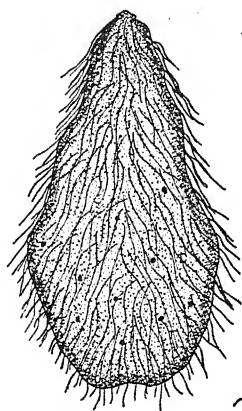
- MALDONADO, JOSÉ F. 1943 A note on the life cycle of *Tamerlania bragai* Santos, 1934. J. Parasitol. 29: 424.
- PENNER, L. R. 1939 *Tamerlania melospizae* n. sp., with notes on the genus. J. Parasitol. 25: 421-424.
- SANTOS, VIOLANTINO 1934 Monostomose renal das aves domesticas. Rev. Dept. Nac. Prod. Animal, Brazil, 1: 203-215.
- SKRJABIN, K. J. 1924 Nierentrematoden der Vögel Russlands. Centr. f. Bakteriöl., II Abt. 62: 80-90.
- YAMAGUTI, S. 1941 Studies on the helminth fauna of Japan. Part 32: Trematodes of birds, V. Jap. J. Zool. 9: 321-341.

## EXPLANATION OF PLATES

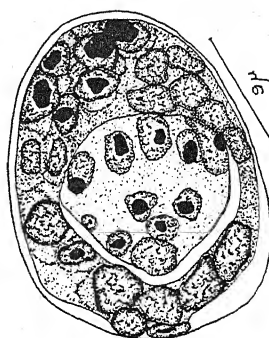
- FIG. 1. The egg.
- FIG. 2. Miracidium obtained from the crop of the snail. Diagrammatic.
- FIG. 3. Daughter sporocyst. Segregation of the first cercarial germ ball and formation of a body cavity. From sections.
- FIG. 4. Daughter sporocyst—diagrammatic. Demonstrates cercarial germ balls and encysted cercariae.
- FIG. 5. The mature cercaria.
- FIG. 6. The encysted cercaria.
- FIG. 7. Excysted metacercaria lying slightly on its right side. Flame cells of the left side shown only.
- FIG. 8. The parasite on the fourth day of life in the kidneys.
- FIG. 9. Eight-day-old stage.
- FIG. 10. Young adult at 2 weeks of age.



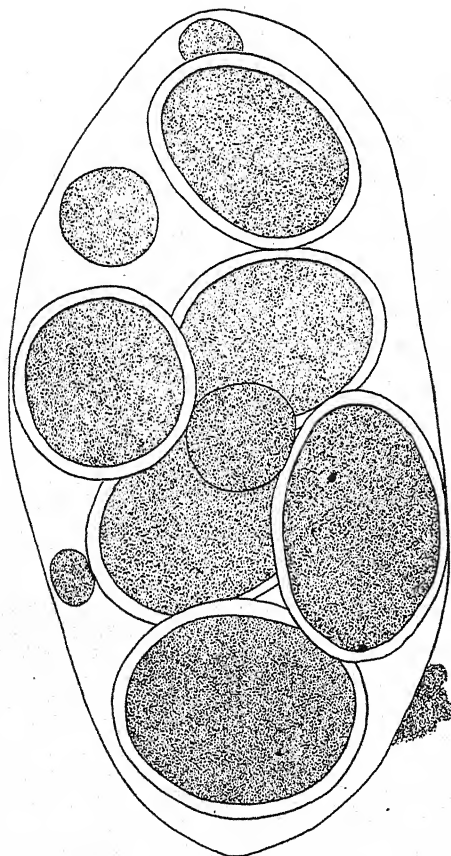
1



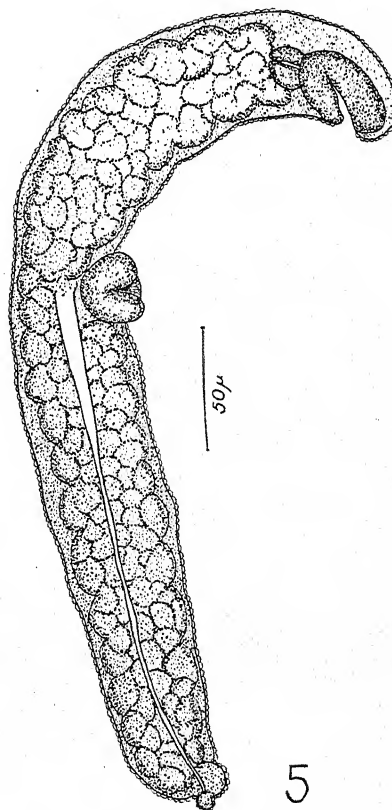
2



3

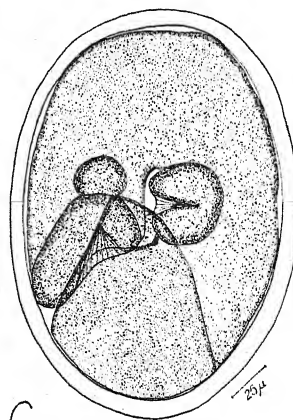
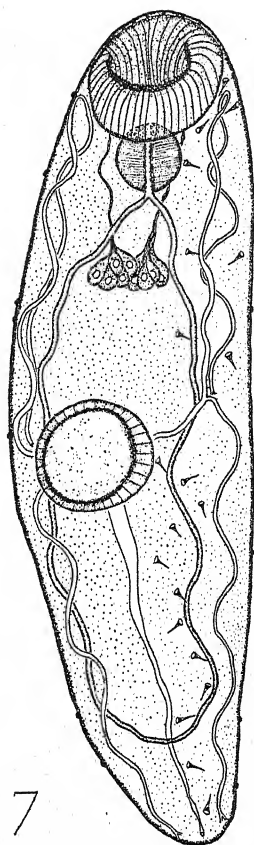


4

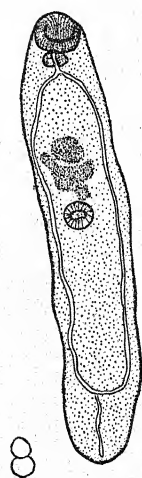


5

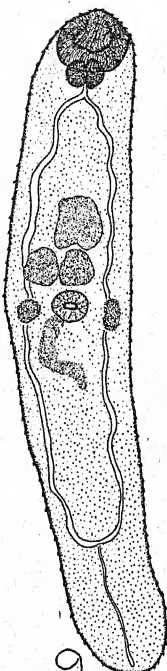




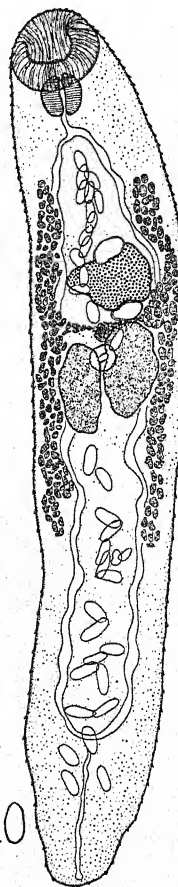
7



8



9



10



## ANOPHELES NATALIAE, A NEW SPECIES FROM GUADALCANAL

JOHN N. BELKIN, Captain SnC, AUS

During a search for breeding places of *Anopheles solomonis* Belkin, Knight and Rozeboom (1945) in the coral foothills of northwestern Guadalcanal a third species in the *lungae* series was discovered and is described in this paper.

### *Anopheles (Myzomyia) nataliae*, n. sp.

**ADULT FEMALE.**—A medium-sized, grayish-brown, speckled anopheline; apical sixth to fifth of labium with a ring of light yellow scales. Length of wing, 3.5–4 mm.

**Head (Fig. 1):** Ornamentation of head and antennae as in *solomonis*. Palpi usually reaching to labella, ornamented as shown in Fig. 1; second and third morphological segments black with narrow ring of white scales at apex, frequently a patch of bronzy scales dorsally in center; fourth and fifth segments each black-scaled basally for a fourth to a third of its length, remainder white, often tinged with yellow; ratio of fourth segment to third segment 1: 1.6–1.7. Labium dark-scaled basally, apical sixth to fifth with a ring of light yellow scales, conspicuously separated from the basal black ventrally, gradually merging into it dorsally; a preapical band of dark scales, often interrupted dorsally.

Labella dull yellow. Buccopharyngeal armature (Fig. 2) of several broad central teeth forming a single row; teeth of similar character, separated by intervals; apices of teeth with many spicules, one of which is elongated, bases with bullae and lateral spines.

**Thorax:** Scutal integument grayish-brown with gray pollinose areas; dark brown eye spots in front of and behind scutal angle; prescutellar space, disc of scutellum and a small area in front of wing root dark brown. White scales on anterior promontory rather short and sparse; central scales elongate anteriorly, posteriorly short and extending one-fourth length of scutum; lateral scales broader, a patch of black scales on humeral angles below lateral tufts. Fossae with several small, broad, recumbent, translucent light scales; long, narrow, whitish scales mixed with whitish hairs in front of wing root. Remainder of scutum and all of scutellum without conspicuous broad scales, vestiture consisting of numerous golden hairs of varying length, some of these appear scale-like under high magnification. Scutal and scutellar bristles light brown. Prothoracic lobes with a large patch of short black scales on upper part. Pleura without scales, integument gray-brown with usual darker lines and spots. Spiracular bristles absent; propleurals 3–5 stout and 2–4 shorter, more slender dark bristles; lower sternopleurals several golden hairs; upper sternopleurals 2–3 dark bristles and variable number of light hairs; prealars 5–10 golden hairs; subalars 5–10 golden hairs; lower mesepimerals absent. Halteres light with dense white scaling on upper part of shaft and all of knob. *Wing* in general as described and figured for *solomonis*; extremely variable; dark sectoral spot (between basal and median dark costal spots) usually reduced, absent, or fused with median dark spot; dark fringe spots frequently more extensive than in *solomonis*. *Legs* speckled, in general as described for *solomonis*.

**Abdomen:** Integument dark grayish-brown. Devoid of scales on tergites and sternites I–VII, vestiture of narrow golden hairs similar to those on scutum. Hairs more numerous on posterior segments, grading into narrow curved scales on segment VIII. Cerci with narrow black scales.

**ADULT MALE.**—In general as in the female. Palpal ornamentation as in *solomonis*. Labium usually entirely dark-scaled. Abdomen as in the female except for numerous yellow scales on tergite VIII. Sidepieces densely covered with yellow scales ventrally and black scales dorsally.

**Genitalia:** Appear to differ from *lungae* in the following respects: phallosome shorter and broader; clubs on claspettes more slender and not as distinctly enlarged at apex; apical hairs of claspettes shorter, only slightly longer than club, inner accessory hairs shorter than club.

**PUPA.**—In general as in *solomonis* with the following exceptions. General coloration brownish-yellow; head shield, mesonotum, and metanotum deep brown; trumpet dark yellowish-brown with a preapical line of deep brown pigmentation. Lateral spines of abd. segment V short, acutely tapered, on the same order as those of III and IV, usually one-third as long as those on VI; lateral spines of VI and VII slender, elongate, acutely tapered, without branches or fraying.

**LARVA.**—Live female larvae are a deep black in color, this intense pigmentation remains particularly in the head capsule, palmate hairs and anal saddle. The male larvae are a dark

reddish-brown except for the black saddle; skins and preserved specimens are also easily recognized by the lighter pigmentation of the head capsule.

**Head** (Fig. 4): Inner clypeal hairs very long, usually with minute lateral barbs; outer clypeals a third to almost half the length of the inner, usually simple, very rarely with minute barbs; distance between outer and inner hairs one-fourth to one-third that between inner hairs; posterior clypeals long, 2-4-branched, sometimes simple, usually reaching tubercles of anterior clypeals, apical portion of hairs very fine. Frontal and subantennal hairs feathered. Antennae with long spines on inner surface; antennal hair simple, minute, situated one-third the distance from the base; terminal hair longer than sabers, 6-10-branched. Occipital hairs short, inner 2-4-branched, outer 2-5-branched. Head capsule in female completely pigmented a deep brown black, in male incompletely yellowish-brown.

**Thorax:** Tubercles of prothoracic hairs 1 and 2 (Fig. 5) fused, very rarely one set may be only approximated; hair 1 with heavy flattened shaft and 18-30 branches, branches usually radiating except at apex which may be extended; hair 2 almost twice as long as 1, 12-20-branched, branches more numerous on outer surface; hair 3 small, simple, arising from the fused tubercles. Prothoracic pleural hairs: 9 long, with 4-8 branches; 10 long, simple; 11 about one-third the long hairs, usually simple, occasionally double; 12 long, simple. Mesothoracic pleural hairs: anterior pair (9, 10) long, simple; hair 11 minute, simple; hair 12 approximately one-fourth of long hairs, simple. Metathoracic pleural hairs: 9 usually simple, occasionally double; 10 long, simple; 11 minute, simple; 12 short, 2-4-branched.

**Abdomen:** Hair 1 on segment I with narrow attenuated, flattened leaflets without serrations, pigmented; hairs 1 on segments II-VII full-sized palmate hairs with broad notched leaflets; 19-25 leaflets on segment III; leaflets (Fig. 3) broad, abruptly narrowed with deep indentations into a delicate terminal filament which is about one-third the length of the shaft; pigmentation heavy and uniform; frequently lateral indentations present on shaft. Hair 2 on segments IV and V simple. Hair 6 of segment IV 2-3-branched; of segment V usually 2, occasionally 3-branched. Anterior tergal plates darkly pigmented, small, half or less the distance between the palmate hairs, except on segment VIII where it is large. Posterior tergal plates very small, present on segments III-VII. Pecten (Fig. 6) with 12-15 teeth, irregularly long, short, and intermediate; larger teeth heavily pigmented, fringed beyond their bases; pecten hair with 6-10 branches. Caudal hooks 6-8. Saddle very heavily pigmented, hair long and simple. Anal gills longer than saddle. Hair 13 of segment V very large, with 8-12 branches.

**Types.**—Holotype ♀, Allotype ♂ with larval and pupal skins, Natalia springs, Poha River Valley, 3 miles south of coast, elevation 200 feet, Guadalcanal, 18 Feb. 1945 (J. N. Belkin). Paratypes: 16 ♀, 6 ♂ with larval and pupal skins, 1 ♂, 18 larvae, same locality as holotype, 10 Dec. 1944, 12-25 Feb. 1945 (J. N. Belkin, M. Cohen, J. J. Cuccio, F. B. Wysocki, E. J. McCormick, Jr.); 2 ♀ with larval and pupal skins, 3 ♀, small tributary of Poha River, 4 miles south of coast, elevation 400 feet, Guadalcanal, 12 Feb. 1945 (J. N. Belkin et al). Holotype and allotype deposited in U. S. National Museum. Paratypes to be deposited in the collections of Council for Scientific and Industrial Research, Canberra, Cornell University and Johns Hopkins School of Hygiene and Public Health.

**Taxonomic position.**—*A. nataliae* is a third member of the *lungae* series in group *Neomysomyia*. It agrees with *lungae* and *solomonis* in palpal, haltere and wing ornamentation and the lack of conspicuous scales on the disc of the scutum in the adult stage, and in the branching of hair 9 of the prothoracic pleural group in the larval stage. The adult female of *nataliae* is separated from the other two species by the extent of light scaling on the labium, the presence of small, broad scales on the fossae, and long narrow scales in front of the wing roots, and the extension of light scales caudad from the central portion of the anterior promontory. The larval stage is immediately recognized by the fused tubercles of prothoracic hairs 1 and 2, the structure of the pecten, the unbranched condition of hair 1 on abdominal segments IV and V, and the extremely heavy pigmentation of the head capsule and anal saddle. In the pupal stage it is intermediate between *lungae* and *solomonis* but can be easily told by the unusual pigmentation of the cephalothorax, trumpets and metanotum, and the structure of the lateral spines on the abdominal segments.

The characters which have been used to separate the species in the *lungae* series are usually clear-cut and quite striking. That these forms are distinct species is

supported by the fact that in a large number of rearings of progenies of wild females of *lungae* and a few *solomonis* no intermediates have been obtained and a remarkable constancy in the specific characters has been observed. Except for the labial ornamentation and some of the features of the pupa the specific characters of *nataliae* are in no way intermediate between *lungae* and *solomonis*, but represent an entirely different line of specialization. The greater extent of scaling of the lateral and anterior portions of the scutum in *nataliae* shows closer relationship to the *punctulatus* series than is exhibited by *lungae* or *solomonis*.

*Biology.*—To date *A. nataliae* has been collected in two areas more than a mile apart in the Poha River valley a short distance downstream from its entrance into the coastal coral foothills from the igneous formations of the Kavo Range. Whether these localities are normal breeding places or represent small colonies established following flushing from breeding places upstream remains to be determined. Both breeding places are seepage and spring areas with clear running water and are densely shaded. The larvae and pupae are very scarce and difficult to collect because of their habit of resting on top of floating vegetation and debris or on the margins of the creeks, usually where the current is fairly swift. The larvae are extremely active and appear to rest normally parallel to an object rather than at right angles to it as is the case with most anophelines. In the laboratory the pupae behave as those of *lungae* and *solomonis* as they usually rest above the water surface and the adults emerge from this position.

*Bironella hollandi*, *A. solomonis*, *farauti* and *lungae* have been collected with *nataliae* in the order of their abundance.

The larvae of *nataliae* in life have the short broad appearance characteristic of the *lungae* series. They differ from *solomonis* and *lungae* in their opaque bodies. The female larvae have coal-black bodies with densely pigmented black head and saddle and are easily distinguished from male larvae which have reddish-brown bodies, dark brown heads, and black saddle.

Adults have not been collected in nature.

#### REFERENCES

- BELKIN, J. N. AND SCHLOSSER, R. J. 1944 A new species of Anopheles from the Solomon Islands. Jour. Wash. Acad. Sci. 34: 268-273. 11 figs.  
BELKIN, J. N., KNIGHT, K. L. AND ROZEBOOM, L. E. 1945 Anopheline mosquitoes of the Solomon Islands and New Hebrides. J. Parasitol. 31: 241-265.

#### EXPLANATION OF PLATE

##### *Anopheles nataliae*, n. sp.

- FIG. 1. Adult female. Head, proboscis and palpus.  
FIG. 2. Adult female. Buccopharyngeal armature.  
FIG. 3. Larva. Leaflet of palmate hair, abd. III.  
FIG. 4. Larva. Anterior portion of head.  
FIG. 5. Larva. Prothoracic submedian group, right side.  
FIG. 6. Larva. Pecten, left side.

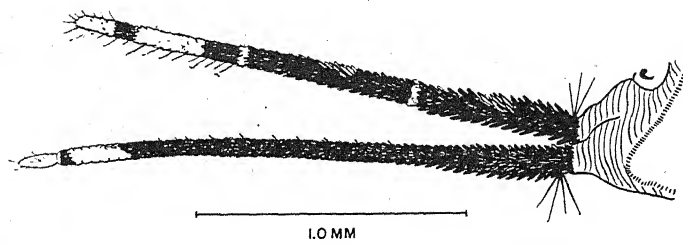


FIG. 1

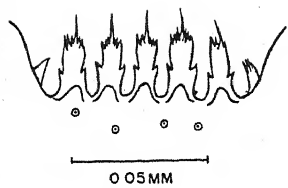


FIG. 2



FIG. 3

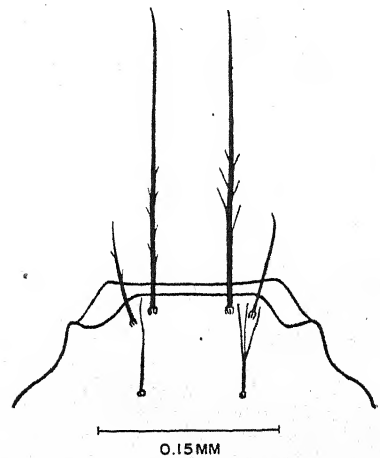


FIG. 4

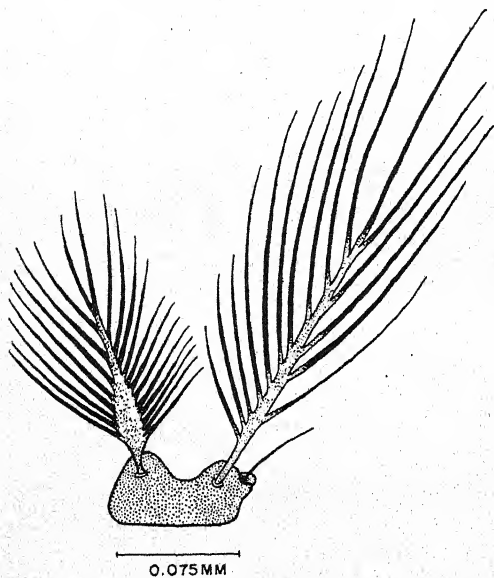


FIG. 5

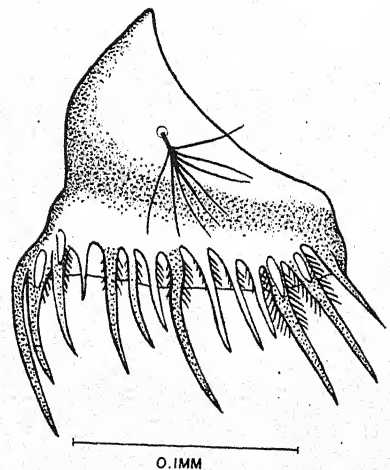


FIG. 6



LABORATORY REARING AND LIFE CYCLE OF *PHLEBOTOMUS*  
(*DAMPFOMYIA*) *ANTHOPHORUS* ADDIS (DIPTERA:  
PSYCHODIDAE)<sup>1</sup>

C. J. ADDIS

The author (1945) described *Phlebotomus (Dampfomyia) anthophorus* from specimens collected at Uvalde, Texas, and published notes on the biology and habits of the adult female in nature. The female flies were captured while feeding on rabbits in the mornings and shipped to Houston, where a simple and practical method of rearing a large number of the flies in the laboratory was employed.

Several methods for rearing sandflies have been described (Whittingham and Rook, 1922; Waterston, 1922; Smith, 1925; Christophers, Shortt, and Barraud, 1926; and Adler and Theodor, 1935), but all of them require much attention in order to maintain the correct amount of moisture. Nájera (1941) used filter paper cups fitted into vials, and moisture supplied by a wick-like arrangement. We tried this method but difficulty was encountered in preventing moisture from condensing on the sides of the vials, which caused the death of the flies. We remedied this somewhat by cutting off the upper end of the vial and covering it with muslin, but the keeping of the flies in individual vials made this method impractical for the rearing of a large number of flies.

In the rearing of sandflies it is necessary to keep the flies at a temperature of 28–29° C and to maintain a high humidity in the breeding chambers without condensation of moisture.

In our laboratory a breeding chamber (Fig. 5) was constructed in which the flies passed through all stages of development without any further change in the apparatus. In preparing the chamber an earthenware saucer 6 inches in diameter and about 1½ inches deep was filled with plaster of Paris to a depth of about 1 inch. A ring of paraffin was poured over the surface of the plaster of Paris except for a space in the center about 3 inches in diameter, and a lantern globe 3½ inches in diameter at the base and 7 inches in height was pressed firmly into the paraffin over this central space. The upper end of the lantern globe was covered with muslin and the entire chamber set in a tray of water. The trays were then placed in an incubator maintained at a temperature of 28° C.

The flies were captured in small vials plugged with cotton moistened with sugar water. For shipping, a cigar box was prepared by cutting several holes in the sides and bottom, and by placing a piece of hardware-cloth about an inch from the bottom. The vials were placed in a wet cloth bag and then spread evenly over the hardware-cloth. Damp cotton was laid over the top of the vials to protect them in transit. On reaching Houston at night, the flies were at once released into a feeding cage a cubic foot in size, closely lined with muslin, and given an opportunity to feed on a hamster enclosed in a hardware cloth cylinder measuring 5 × 3 × 3 inches.

---

Received for publication, May 23, 1945.

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Rice Institute, Houston, Texas. The author wishes to express his appreciation to Dr. Asa C. Chandler for his advice and aid, and to Mrs. Evelyn Hake for the preparation of the slides.



When examined between 8 and 9 AM very few flies had fed, but by noon practically every fly which showed no signs of a previous blood meal had fed. The feeding of the caged flies during the morning coincides with the observations of the writer (1945) on the adults in nature. The females, after engorging, were placed in the breeding chambers.

After oviposition the eggs were counted and food for the larvae was spread on the surface of the plaster of Paris. The first food mixture we tried consisted of dried rabbit feces and blood, but the mature larvae failed to pupate over a period of 2 months. When a few drops of fluid containing all the known components of the vitamin B complex were added to the food, pupation occurred within 3 days. Later we tried a mixture of rich garden earth and rabbit blood, which gave satisfactory results without the addition of vitamins, and was finally used exclusively. This medium was prepared by adding rabbit blood to the finely ground garden earth until it had the consistency of a thick paste. The mixture was then dried, ground into a powder, and kept in glass jars until used. The food material was spread unevenly over the surface of the plaster of Paris so that there was a gradation from very moist areas to practically dry areas. The correct amount of moisture can easily be judged by looking at the medium under a binocular; if a film of water is observed, the medium is too wet and should be corrected by increasing the thickness of the plaster of Paris in the saucer. A mold and small mites appeared in some of the saucers, but apparently had no harmful effect on the development of the flies.

After pupation the chambers were watched closely for the appearance of adults. These were at once transferred to another chamber with a piece of cotton soaked with sugar water on the floor. After 2 to 3 days the flies were released into a feeding cage and allowed to take a blood meal. After feeding the flies were put back into the breeding chambers and left until oviposition or death.

This method proved highly efficient in our work with *Phlebotomus anthophorus* and could probably be adapted to the rearing of other species of sandflies. However, when the same procedure was used for the rearing of *P. diabolicus*, the larvae, although they grew to maturity satisfactorily, failed to pupate, even when a mixture of the known B vitamins was added to the food. It would appear from this, that the accessory food factors required for pupation vary with different species of *Phlebotomus*.

#### LIFE CYCLE

The following observations on the life cycle of *Phlebotomus anthophorus* were made on 6 different groups of 12 flies kept at a temperature of 28 to 29 degrees C and at a constant humidity. The food for the larvae consisted of the garden earth and rabbit-blood mixture described above. The average time for each developmental stage is given in parenthesis.

The engorged females, kept in the breeding chambers and allowed to feed on sugar water, oviposited within 3 to 6 (4.7) days after taking a blood meal. The eggs were deposited on the surface of the plaster of Paris in clusters of 5 or 6, or singly, with as many as 20 eggs being deposited by one female. The freshly deposited eggs are a light tan in color, turning a dark brown after a few hours, with irregular light striations over the surface (Fig. 1). The eggs are elongate, somewhat smaller at one end, and measure 373-403 (381) microns in length and 119-134 (126) microns in the greatest width.

The incubation periods ranged from 9 to 14 (10.5) days. The newly hatched larvae are white in color and have 2 caudal setae. After the first molt the larvae turn darker in color, having a dirty appearance, and have 4 caudal setae (Figs. 2 and 3). The larvae were observed feeding on the medium and moving about slowly in a characteristic looping movement. While young they favored the more moist areas of the medium, but towards the end of the larval stage they migrated towards the drier places to pupate.

Pupae (Fig. 4) appeared within 22 to 38 (28.4) days after the hatching of the larvae, and adults emerged within 8 to 10 (8.7) days after pupation. The developmental period from the laying of the eggs to the emergence of the adults required 40 to 60 (49) days.

The females took blood meals within 2 to 4 (3) days after emerging and lived for 4 to 9 (6) days in the breeding chambers after the blood meal. During this time the flies were maintained on raisins, and one fly took a second blood meal on the eighth day. The total length of life for the emerged females was 8 to 11 (9.2) days and for the males 4 to 8 (5.3) days.

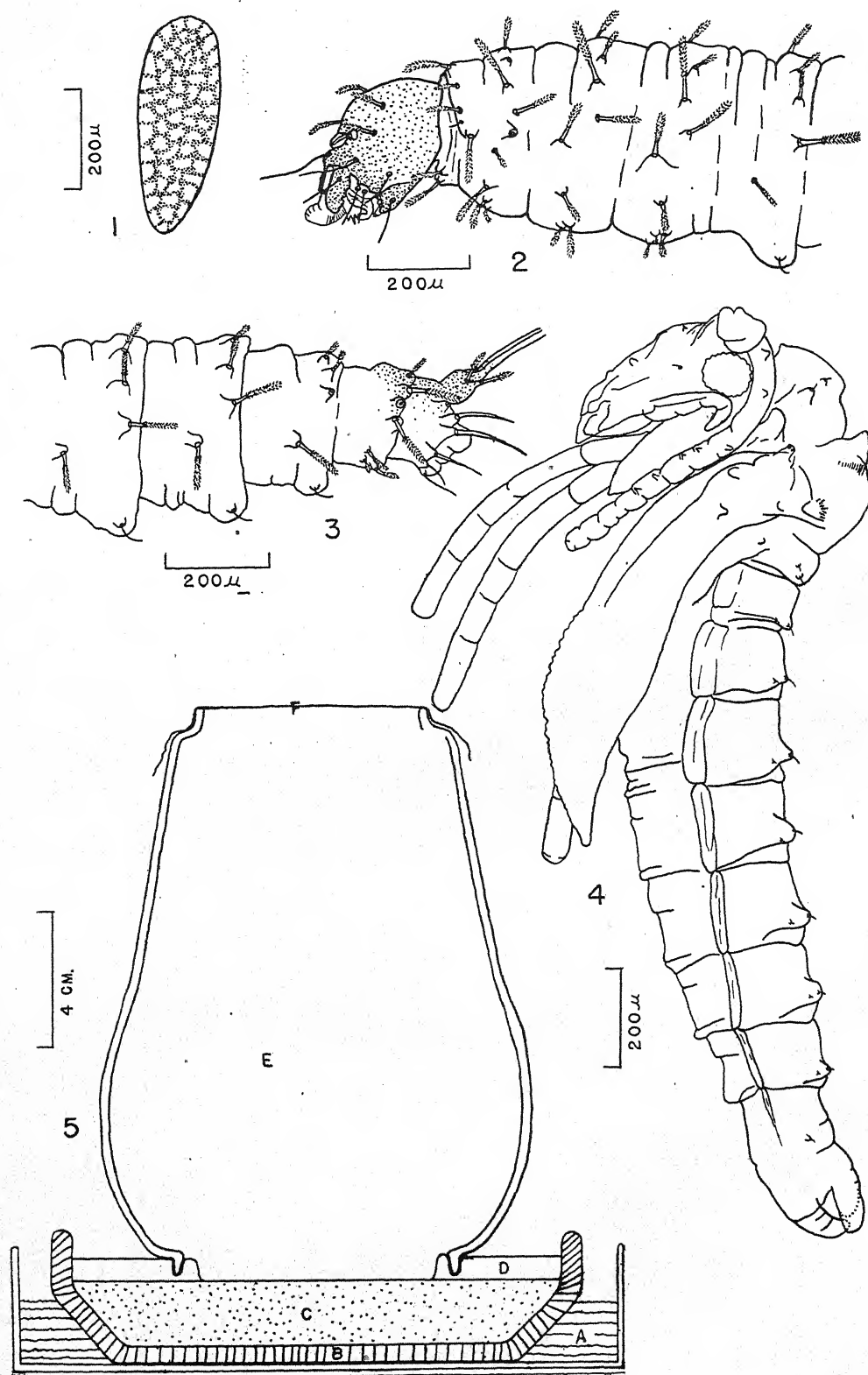
#### REFERENCES

- ADDIS, C. J. 1945 *Phlebotomus (Dampfomyia) anthophorus*, n. sp., and *Phlebotomus diabolicus* Hall from Texas (Diptera: Psychodidae). J. Parasitol. 31: 119-127.
- ADLER, S. AND THEODOR, O. 1935 Investigations on Mediterranean kala-azar. VIII. Further observations on Mediterranean sandflies. Proc. Roy. Soc. B 116: 505-515.
- CHRISTOPHERS, S. R., SHORTT, H. E. AND BARRAUD, P. J. 1926 Technique employed in breeding *Phlebotomus argentipes* in Assam. Ind. Med. Res. Memoirs No. 4: 173-176.
- NÁJERA, L. 1941 Descripción de un dispositivo nuevo para la cría de *Phlebotomus*. Rev. Med. Trop. y Parasit. 7: 8-12.
- SMITH, R. O. A. 1925 A note on a simple method of breeding sandflies. Ind. J. Med. Res. 12: 741-742.
- WATERSTON, J. 1922 A contribution to the knowledge of the bionomics of sandflies. Ann. Trop. Med. and Parasitol. 16: 69-92.
- WHITTINGHAM, H. E. AND ROOK, A. F. 1922 Demonstration of the life history of *Phlebotomus papatasi* and its maintenance in captivity. Tr. Roy. Soc. Trop. Med. and Hyg. 16: 262-266.

#### EXPLANATION OF PLATE

##### *Phlebotomus (Dampfomyia) anthophorus* Addis

- FIG. 1. Egg.
- FIG. 2. Head and thorax of fourth stage larva.
- FIG. 3. Posterior end of fourth stage larva.
- FIG. 4. Pupa.
- FIG. 5. Diagram of breeding chamber.
- A. Water
  - B. Earthenware saucer
  - C. Plaster of Paris
  - D. Paraffin
  - E. Lantern globe
  - F. Muslin



# THE GENUS *DISPHARYNX* (NEMATODA: ACUARIIDAE) IN GALLIFORM AND PASSERIFORM BIRDS

FRANS C. GOBLE AND H. L. KUTZ

Game Research Center, New York State Conservation Department, Delmar, New York

Commonly listed as a parasite of poultry (Mönnig, 1938; Barger and Card, 1938; Wehr, 1943) is the proventricular worm *Dispharynx spiralis* (Molin, 1858) Skrjabin, 1916. Parasitological examinations of poultry in North America, however, have shown that its presence in domestic birds in this part of the world is rare.

Autopsies of certain wild gallinaceous birds, on the other hand, have revealed relatively high incidence of the parasite. Its demonstrated presence in several species of perching birds and a comparative study of the form from several different hosts has pointed to the possibility that most of the specimens of *Dispharynx* from galliform and passeriform birds in both hemispheres belong to the same species. It is the purpose of this article to record observations on the proventricular worms of this genus which we have encountered and to discuss the probable identity of the forms reported from the above mentioned birds.

## HISTORICAL

Rudolphi (1819) described *Spiroptera nasuta* from the proventriculus of the house-sparrow (*Passer domesticus*) from Vienna. In 1844 ("1845"), Dujardin encountered the same form in sparrows in Paris. He erected the genus *Dispharagus* and included Rudolphi's form in it, as *D. nasutus* (Rud. 1819). Since *Dispharagus* was a renaming of a group of species for which two generic names were already in existence, and included the type of the genus *Acuaria* Bremser, 1811, it subsequently fell into synonymy with the latter genus (see Cram 1927a).

Diesing (1851) reported *S. nasuta* from domestic fowl (*Gallus gallus*) in Austria. Subsequently Casali (1874), Colucci (1893), and Piana (1897) recorded it from fowl in Italy; LeGros (1864), Neumann (1892), and Rabieaux (1900) from France; Johnston (1912), from Australia; Purvis (1913) from Washington and Oregon; Ransom (1916) from Guam as *Cheilospirura nasuta*. It has also been listed from Belgian Congo (Cram 1927a).

Molin (1858) found proventricular worms in the domestic fowl in Italy which he described as a new species, *Dispharagus spiralis*. Also recorded as *D. spiralis* were worms from Guinea fowl (*Numida meleagris*) in Italy (Stossich, 1891) and pigeon (*Columba livia*) in Tunisia (Bridré, 1910).

In 1912 Railliet, Henry and Sisoff, revised the genus *Acuaria* Bremser, 1811, erecting the subgenus *Dispharynx*, with *A. (D.) nasuta* (Rud., 1819) as the type species, including also *A. spiralis* (Molin, 1858) and three species from falconiform birds. Without giving their reasons they expressed the opinion that all the records of *A. nasuta* from galliform and columbiform birds were in reality reports of *A. spiralis*. They may have been influenced by the fact that the only records of *Dispharynx* in passeriform birds to that date were those of Rudolphi (1819) and Dujardin (1844).

Received for publication, May 5, 1945.



Seurat (1916) recorded *A. spiralis* from a partridge (*Alectoris barbara*) in Algeria, and listed (without details or reference) the pheasant as a host of the parasite. In the same year Skrjabin, who raised the sub-genus *Dispharynx* to generic rank, recorded it from chickens in Russian Turkestan. Recent old world reports are those of Gushanskaia (1937) who found it in *Coracias garrula* in Uzbekistan and Kazakhstan, and Madsen (1941) who encountered it in partridges (*Perdix perdix*) and pheasants (*Phasianus colchicus*) in Denmark.

Since Walton (1923) reported *A. spiralis* in this country from the bronzed grackle (*Quiscalus versicolor*) in Illinois, the form commonly designated as *Dispharynx spiralis* (Molin, 1858) Skrjabin, 1916 has been reported from a number of localities and hosts in North America.

Its pathogenicity for the Ruffed Grouse (*Bonasa umbellus*) was first pointed out by Allen (1924) who described and pictured the proventriculitis caused by its presence. It has been listed from this host in New York (Allen, 1924), Maine and Connecticut (Gross, 1925), Massachusetts, Rhode Island and New Jersey (Allen and Gross, 1926), New Hampshire (Gross, 1930), Nova Scotia (Gross, 1931), and Michigan (Fisher, 1939).

The parasite has also been reported from several other game birds: sharp-tailed grouse (*Pedioecetes phasianellus*) in Wisconsin (Gross 1931); bobwhite quail (*Colinus virginianus*) in Virginia (Cram, 1931a), North Carolina (Moore, 1933) and Ohio (Venard, 1933); Hungarian partridge (*Perdix perdix*) in Wisconsin (Cram, 1931b) and Ohio and Michigan (Yeatter, 1934); ringnecked pheasant (*Phasianus colchicus*) and golden pheasant (*Chrysolophus pictus*) in New York (Goble and Cheatum, 1943).

To our knowledge the only recent records from domesticated galliformes in North America are those of Cram (1927b, 1932c); Guinea fowl (*Numida meleagris*) from Puerto Rico; domestic fowl (*Gallus gallus*) from Louisiana; turkey (*Meleagris gallopavo*) from Maryland. There are, however, in addition to Walton's record from the bronzed grackle, a number of reports of this nematode from passeriform birds: house sparrow (*Passer domesticus*) from the District of Columbia (Cram, 1932a); robin (*Turdus migratorius*) from New Jersey (Cram 1932a) and New York (Webster 1943); catbird (*Dumetella carolinensis*) from New Jersey (Cram 1932b); Carolina wren (*Thryothorus l. ludovicianus*) from Texas (Harwood, 1933). Both Cram (1928) and Wehr (1943) have observed the parasite in pigeons (*Columba livia*) from Texas and California, respectively.

During the decade 1932-1941 inclusive, over 2800 specimens of ruffed grouse (*Bonasa umbellus*) were examined in the Pathological Laboratory, Bureau of Game, New York State Conservation Department. The results of these examinations were recorded by Levine and Goble (Unpublished MS, prepared 1942). Their salient conclusions may be summarized briefly: (1) In the areas in which it occurs, *Dispharynx* is the most important helminth parasite of ruffed grouse. (2) It occurs in about one-third of the birds-of-the-year examined during the fall and winter. During the same seasons, only about one-seventh of the adults are infected. (3) The proventriculitis which usually accompanies the infections is often severe and sometimes fatal. (4) The parasite has not been found in the Adirondack region in New York and is apparently absent from the northern forests in other states as well, although Oniscoidea, the only reported intermediate hosts (Cram, 1931b) are present in those areas.



## MATERIALS

The hosts examined for proventricular worms are listed in Table 1. The galliforms were submitted to the laboratory at various times between 1938 and 1945. The small numbers of bobwhite quail and Hungarian partridge examined are a reflection of their relative scarcity in most of upper New York State. All birds listed were from the wild with the exception of the peafowl which was a domestic inhabitant of the Union College Campus at Schenectady.

The passeriform birds were collected by various means during the period of March through December, 1944. Some were shot, some were trapped, others were victims of predation or accidental trauma. The species collected represent a

TABLE 1.—Occurrence of *Dispharynx* in birds in New York

Hosts	Juveniles			Adults		
	Examined	Infected	Per cent	Examined	Infected	Per cent
Galliformes						
Ruffed grouse ( <i>Bonasa umbellus</i> )	301	102	34	303	40	13
Ringnecked pheasant ( <i>Phasianus colchicus</i> )	203	2	1	937	0	0
Peafowl ( <i>Pavo cristatus</i> )	0	0	0	1	1	100
Hungarian partridge ( <i>Perdix p. perdix</i> )	9	2	22	33	2	7
Eastern bobwhite ( <i>Colinus v. virginianus</i> )	0	0	0	2	1	50
Passeriformes						
Eastern crow ( <i>Corvus b. brachyrhynchos</i> )	12	2	18	25	0	0
Catbird ( <i>Dumetella carolinensis</i> )	1	1	100	1	0	0
Eastern robin ( <i>Turdus m. migratorius</i> )	20	6	30	55	1	2
Eastern bluebird ( <i>Sialia s. sialis</i> )	5	2	40	31	4	13
Starling ( <i>Sturnus v. vulgaris</i> )	40	4	10	78	2	3
House sparrow ( <i>Passer d. domesticus</i> )	23	1	4	27	0	0
Eastern cowbird ( <i>Molothrus a. ater</i> )	18	5	28	48	4	8

The following numbers of other passeriform birds have been examined for *Dispharynx* and found negative: Bobolink (*Dolichonyx oryzivorus*)—20; eastern meadowlark (*Sturnella m. magna*)—24; eastern redwing (*Agelaius p. phoeniceus*)—39; bronzed grackle (*Quiscalus versicolor*)—44; slate-colored junco (*Junco h. hyemalis*)—22; eastern tree sparrow (*Spizella a. arborea*)—20; eastern song sparrow (*Melospiza m. melodia*)—26.

haphazard sample of the common forms which occur in the vicinity of several game farms and refuges in upper New York State. One specimen, the catbird, was found sick and picked up by hand.

Birds listed as juveniles were those in which the *bursa fabricii* was still present. No passeriforms were collected after December, so probably most of the juveniles were less than 6 months old. Grouse, however, were taken during all months of the year and since the *bursa* persists into the winter in grouse, some of the birds recorded as juveniles were as old as 9 months.

Specimens of *Dispharynx* collected from the proventriculi of the listed species of birds were fixed in hot 70 per cent alcohol with 5 per cent glycerin, and were examined either in glycerin, after the evaporation of the alcohol, or in lactophenol. The number of worms available for morphological study is indicated in Table 3. Not listed individually therein are the infections in ruffed grouse, from which abundant *Dispharynx* material was obtained.

## INCIDENCE

The incidence of *Dispharynx* in the galliform and passeriform birds examined is indicated in Table 1. To our knowledge, proventricular worms of this genus have not previously been reported in the peafowl, crow, bluebird, starling or cowbird. In pheasants, where the occurrence was extremely rare, the only infections were in chicks from one limited area.

The large number of ruffed grouse examined permitted a more detailed breakdown of the data as to age and season. These data are presented in Table 2. In the collection of grouse chicks from three summers, the earliest date on which *Dispharynx* infection was observed was July 20 and the smallest (youngest) bird in which it appeared weighed 144 grams (about 5 weeks old). Eliminated from the data used in determining incidence, therefore, were all grouse weighing less than 144 grams or taken before July 20th as well as those taken in areas where large collections have revealed no infections and where the parasite apparently is

TABLE 2.—Incidence of *Dispharynx* infection in grouse, indicating ages of birds, seasons and fatality of infections

Ages of birds	Season	Number examined	Number infected	% infected	Number fatalities	% of infected cases fatal
Juveniles (less than 3 mo. old)	Summer	115	35	30.4	1	
Juvenile (3-6 mo. old) . . . . .	Fall	158	61	38.6	26	
Juvenile (6-9 mo. old) . . . . .	Winter	28	6	21.4	4	
Total . . . . .		301	102	33.8	31	30.3
Adult (over 9 mo.) . . . . .	Spring	56	8	14.3	5	
Adult (over 12 mo.) . . . . .	Summer	90	7	7.8	2	
Adult (over 15 mo.) . . . . .	Fall	98	15	15.3	2	
Adult (over 18 mo.) . . . . .	Winter	59	10	16.8	4	
Total . . . . .		303	40	13.2	13	32.5

not enzootic in grouse (i.e., the Adirondack region). Thus the figures given are a good estimate of the incidence to be expected in birds old enough to be infected in parts of the state where the parasite is known.

The incidence in juvenile grouse is significantly greater than in adults in both the summer and fall seasons, but the fluctuations in incidence from season to season are not significant. Higher susceptibility of young animals to infection is a generally accepted principal in parasitology, but little seems to have been recorded on this subject with reference to *Dispharynx* infections. Madsen (1941) observed that in partridges in Denmark the parasite "was encountered most often and in the largest numbers in young adult birds," and that in pheasants *Dispharynx* "appeared only in chicks and the infections were light." The available information on the occurrence of the worm in passeriforms seems to indicate the same differences in incidence due to age of host.

## SIZE OF INFECTIONS

The number of worms encountered in infected birds is shown in Table 3. Omitted from this table are the records of worms from grouse, which are so numerous as to require an inordinate amount of space; the information can be adequately presented as averages. The average number found in both juvenile and adult grouse in which no gross proventriculitis was observed was 9. The

numbers occurring in infections with proventriculitis ranged from 10 to 246. Further information on this group appears in the section on Pathology below.

Some observations on size of infections made by other authors may be given for comparison. The greatest number observed in ruffed grouse by Allen and Gross (1926) was 228. Cram (1931a) reported 17 in a quail though Venard's (1933) heaviest infection was 4. Webster (1943) found 150 worms in a robin. Gushanskaia (1937b) found 146 in a specimen of *Coracias garrula*. Cram (1931b) reported 230 in a catbird, and 4 in the house sparrow.

TABLE 3.—Numbers of worms encountered in *Dispharynx* infections

Species of bird	Age of bird	Number of worms	
		Male	Female
Ringnecked pheasant . . . .	Juvenile	19	17
" " . . . .	Juvenile	2	4
Peafowl . . . . .	Adult	1	1
Hungarian partridge . . . .	Juvenile	2	1
" " . . . .	Juvenile	1	2
" " . . . .	Adult	1	1
" " . . . .	Adult	1	1
Eastern bobwhite . . . . .	Adult	1	1
Eastern crow . . . . .	Juvenile	1	2
" " . . . . .	Juvenile	1	1
Catbird . . . . .	Juvenile	117	104
Eastern robin . . . . .	Juvenile	10	17
" " . . . . .	Juvenile	0	1
" " . . . . .	Juvenile	12	5
" " . . . . .	Juvenile	2	5
" " . . . . .	Juvenile	4	4
" " . . . . .	Juvenile	1	1
" " . . . . .	Adult	4	3
Eastern bluebird . . . . .	Juvenile	2	2
" " . . . . .	Juvenile	10	2
" " . . . . .	Adult	1	1
" " . . . . .	Adult	2	2
" " . . . . .	Adult	1	1
" " . . . . .	Adult	18	20
Starling . . . . .	Juvenile	1	1
" " . . . . .	Juvenile	2	1
" " . . . . .	Juvenile	2	2
" " . . . . .	Juvenile	2	2
" " . . . . .	Adult	1	0
" " . . . . .	Adult	0	2
House sparrow . . . . .	Juvenile	2	3
Eastern cowbird . . . . .	Juvenile	2	3
" " . . . . .	Juvenile	10	15
" " . . . . .	Juvenile	19	12
" " . . . . .	Juvenile	1	2
" " . . . . .	Juvenile	5	7
" " . . . . .	Adult	1	2
" " . . . . .	Adult	6	7
" " . . . . .	Adult	5	7
" " . . . . .	Adult	3	5
" " . . . . .	Adult	2	1

It will be noted that among the 32 infected passeriforms 10 harbored equal numbers of male and female parasites (from 1 to 4 of each sex), 14 had more females than males, and 8 had more males than females, although the total indicates a sex ratio of practically 50:50 (248 males, 243 females). This is in contrast to the situation which prevails in ruffed grouse. In a group of 15 grouse, in which the worms in each bird were sexed, the females outnumbered the males in 13 instances and the sex ratio of the worms as determined by the total was 42:58 (436 males, 614 females). No explanation for this difference in sex ratio of the parasites in different hosts is apparent.

## PATHOLOGY

Pathological changes attributable to *Dispharynx* infections range from micro-

scopic leucocytic infiltration and congestion at the point of attachment of the worms to severe chronic catarrhal proliferative proventriculitis in which the saccular stomach is enlarged to several times its normal size and the lumen is plugged with a necrotic mucous mass of sloughed and sloughing epithelium.

In general, the degree of inflammation of the proventriculus was observed to be proportional to the size of the infections. This is confirmed by data from examinations of ruffed grouse. On autopsy, the degree of proventriculitis, when present, was recorded as slight, moderate or severe (+, ++, +++). The total number of worms present was also recorded for each examination. Of 30 grouse for which these data were available 10 showed slight proventriculitis, 10 moderate, and 10 severe. The number of worms found in the first group ranged from 10 to 27 (average 18); in the second group 18 to 109 (average 50); in the third group 70 to 246 (average 132).

At post-mortem examination of the 604 ruffed grouse listed, an estimation was made as to the probability of fatality of the *Dispharynx* infections observed, regardless of the immediate cause of death. Some of the birds were actually found dead in the wild, some were sick and readily taken alive, subsequently dying in captivity; others were caught by hunting dogs or predators, still others were killed by automobiles or shot by hunters and collectors.

Of the 102 infected juvenile grouse 31 (30.3 per cent) either died as a result of the infection or were considered seriously enough affected so that death would have resulted soon had they not been collected by one of the above-mentioned means. Of the 40 infected adults 13 (32.5 per cent) were considered to be fatally infected. No similar information could be adduced for the passerine birds. The catbird was the only one collected which was obviously adversely affected by its *Dispharynx* infection.

#### MORPHOLOGY

Morphological examination of specimens of *Dispharynx* from the species listed revealed their identity with the form commonly known as *Dispharynx spiralis* (Molin, 1858) Skrjabin, 1916. Measurements on the worms all fell within the limits indicated by Cuvillier's (1937) studies and it is felt that little purpose would be served by amplification of her table of measurements.

The vulva was, in all cases, in the posterior part of the body, as described by Molin (1858) for *D. spiralis* and by Piana (1897) for *D. nasuta*. Since the position of the vulva is the only character consistently proposed for the separation of the above-mentioned species, the situation prevailing in all of our material from both passeriform and galliform birds is mentioned here in anticipation of the discussion which follows concerning the probable conspecificity of the two species.

#### DISCUSSION

The conclusion that all the forms of *Dispharynx* recently recorded from galliform, columbiform and passeriform birds in the Western Hemisphere are conspecific leads to the consideration of the identity of the helminths reported as *Dispharynx nasuta* (Rudolphi, 1819) Stiles and Hassall, 1920, originally described from the house sparrow in Vienna and later recorded from chickens in Europe, Australia, and North America.



A survey of the literature on *Dispharynx* reveals that the only substantial character proposed to distinguish *D. spiralis* from *D. nasuta* is the position of the vulva. Rudolphi's (1819) description did not indicate whether the vulva was located in the anterior or posterior part of the body. Dujardin (1844), however, stated its position to be anterior and his description has been followed by many subsequent authors, none of whom, it is apparent, ever observed at first hand *Dispharynx* material from passerine birds (Molin, 1860; Stossich, 1891; Neveu-Lemaire, 1912).

When Diesing (1851) reported *Spiroptera nasuta* from the chicken for the first time he did not mention the position of the vulva, but he did place a question mark after the name *Dispharagus nasutus* Dujardin which may indicate that he questioned some part of Dujardin's description in the light of his material. Subsequently Molin (1858) found a *Dispharynx* in the chicken and described it as *D. spiralis*, indicating the position of the vulva to be in the posterior part of the body.

Apparently the only investigator who has reported *Dispharynx* females with the vulva anterior is Dujardin and there is no modern work indicating this morphological character in worms of this genus from passiformes, galliformes or columbiformes. Yorke and Maplestone (1926) give an original figure of *D. nasuta* showing the vulva to be posterior. In our opinion, it is highly probable that Dujardin's observations on this point were erroneous.

The records of *D. spiralis* from the type host of *D. nasuta*, the cosmopolitan distribution of both species, and their morphological identity seem to be legitimate reasons for considering *Dispharynx spiralis* (Molin, 1858) Skrjabin, 1916 to be a synonym of *Dispharynx nasuta* (Rudolphi, 1819) Stiles & Hassall, 1920, which has priority by 39 years.

*Dispharynx nasuta* (Rudolphi, 1819) Stiles & Hassall, 1920

Synonyms: *Spiroptera nasuta* Rudolphi, 1819; *Dispharagus nasutus* (Rudolphi, 1819); Dujardin, 1844; *Dispharagus spiralis* Molin, 1858; *Filaria nasuta* (Rudolphi, 1819) Schneider, 1866; *Dispharagus tentaculatus* Colucci, 1893; *Dispharagus spiralis columbae* Bridré, 1910; *Acuaria (Dispharynx) nasuta* (Rudolphi, 1819) Railliet, Henry & Sisoff, 1912; *Acuaria (Dispharynx) spiralis* (Molin, 1858) Railliet, Henry & Sisoff, 1912; *Cheilospirura nasuta* (Rudolphi, 1819) Ransom, 1916; *Dispharynx spiralis* (Molin, 1858) Skrjabin, 1916; *Dispharynx stonae* Harwood, 1933.

Hosts: Passeriformes: *Passer domesticus* (type host), *Corvus brachyrhynchos*, *Coracias garrula*, *Thyrothorus ludovicianus*, *Dumetella carolinensis*, *Turdus migratorius*, *Sialia sialis*, *Sturnus vulgaris*, *Quiscalus versicolor*, *Molothrus ater*. Galliformes: *Meleagris gallopavo*, *Numida meleagris*, *Pavo cristatus*, *Perdix perdix*, *Alectoris barbara*, *Colinus virginianus*, *Phasianus colchicus*, *Gallus gallus*, *Chrysolophus pictus*, *Bonasa umbellus*, *Pedioecetes phasianellus*. Columbiformes: *Columba livia*.

Geographical Distribution: Europe: Austria (type locality), France, Italy, Spain, Denmark. Asia: Russian Turkestan, Uzbekistan, Kazakhstan. Africa: Tunisia, Algeria, Belgian Congo. North America: Puerto Rico, United States (New England, New York, New Jersey, Maryland, District of Columbia, Virginia, Ohio, Michigan, Illinois, Wisconsin, Louisiana, Texas, California), Canada (Nova Scotia). South America: Brazil. Australia. Guam.

## SUMMARY

The incidence of proventricular nematodes of the genus *Dispharynx* in galliform and passeriform birds in New York State is given. Records from peafowl, crow, bluebird, starling, and cowbird are probably new. Data indicate significantly higher incidences in juveniles than in adults.

Observations are made on the size of infections and the pathologies attributable to the presence of the parasites. Tissue changes were, in general, proportional to the size of the infections. A little less than a third of the ruffed grouse examined were considered to have died as a result of parasitosis by *Dispharynx* or were so seriously affected that death was inevitable.

Morphologic and taxonomic features of the parasites are discussed and reasons set forth for considering *Dispharynx spiralis* (Molin, 1858) Skrjabin, 1916, to be a synonym of *Dispharynx nasuta* (Rudolphi, 1819) Stiles and Hassall, 1920. Synonymy, host records and geographical distribution are given.

## REFERENCES

- ALLEN, A. A. 1924 The grouse disease. *Am. Game* 13: 12-14.  
 ————AND GROSS, A. O. 1926 Ruffed grouse investigation, season of 1925-26. *Am. Game* 15: 81-84, 86.  
 BARGER, E. H. AND CARD, L. E. 1938 Diseases and parasites of poultry. 2nd Ed. Lea and Febiger, Phila. 386 pp. (pp. 299-300).  
 BRIDRÉ, J. 1910 Un disparage du pigeon. *Bull. Soc. Path. Exot.* 3: 38-39.  
 CASALI, T. 1874 Nuova varietà di Spiroptera del pollo domestico. *Ann. Soc. Nat. Modena* 8: 1-12.  
 COLUCCI, V. 1893 Enzoozia verminosa dei polli prodotta dal *Dispharagus nasutus* Rud. *Mem. R. Accad. Sc. Ist. Bologna* 3: 605-617.  
 CRAM, E. B. 1927a Bird parasites of the Nematode suborders Strongylata, Ascaridata, and Spirurata. *U. S. Nat. Mus. Bull.* 140: 237-244.  
 ————1927b (New records of distribution for various nematodes). *J. Parasitol.* 14: 70.  
 ————1928 Nematodes of pathological significance found in some economically important birds in North America. *U. S. Dept. Agric. Tech. Bull.* 49: 10 pp.  
 ————1931a Nematodes (Roundworms) in quail. In Stoddard's "Bobwhite quail." Charles Scribner's Sons, N. Y. pp. 240-296.  
 ————1931b Developmental stages of some nematodes of the Spiruroidea parasitic in poultry and game birds. *U. S. Dept. Agric. Tech. Bull.* 227: 27 pp.  
 ————1932a New host records for *Dispharynx spiralis*. *J. Parasitol.* 18: 303.  
 ————1932b Additional observations on bird hosts of *Dispharynx spiralis*. *J. Parasitol.* 18: 310.  
 ————1932c Recent findings in connection with parasitism of game birds. *Tr. Am. Game Conf.* 18: 243-247.  
 CUVILLIER, E. 1937 Observations on the biological and morphological relationships of *Dispharynx spiralis* in bird hosts. *Rabot. Gel'mint. (Skrjabin)*. pp. 99-104.  
 DIESING, K. M. 1851 *Systema helminthum*. Vindobonae. p. 212.  
 DUJARDIN, F. 1844 ("1845") *Histoire naturelle des helminthes*. Paris. p. 75.  
 FISHER, L. W. 1939 Studies of the eastern ruffed grouse in Michigan (*Bonasa umbellus umbellus*). *Mich. Agric. Exper. Sta. Tech. Bull.* 166: 33-34.  
 GOBLE, F. C. AND CHEATUM, E. L. 1943 *Dispharynx spiralis* in golden and ringnecked pheasants in New York. *J. Parasitol.* 29: 230-231.  
 GROSS, A. O. 1925 Diseases of the ruffed grouse. *Science* 62: 55-57.  
 ————1930 Report of New England ruffed grouse investigations. *New England Game Conf.* pp. 58-62.  
 ————1931 Report of the New England ruffed grouse investigation and Wisconsin prairie chicken investigation. *New England Game Conf.* pp. 44-59.  
 GUSHANSKAIA, L. Kh. 1937 K faune nematod *Coracias garrula* v SSSR. *Rabot. Gel'mint. (Skrjabin)*. pp. 215-220.  
 HARWOOD, P. D. 1933 Some spiruroid nematodes from Texas birds. *Tr. Am. Micr. Soc.* 52: 173-176.

- JOHNSTON, T. H. 1912 Notes on some Entozoa. *Proc. Roy. Soc. Queensland* 24: 63-91.
- LEGROS, C. 1864 Affection vermineuse insolite chez les gallinacés. *Compt. Rend. Soc. Biol.* 5: 218-219.
- LEVINE, P. P. AND GOBLE, F. C. 1942 Parasitism and disease in ruffed grouse in New York state. In "Report of New York Ruffed Grouse Investigation," edited by G. Bump. (Unpublished M.S.)
- MADSEN, H. 1941 The occurrence of helminths and coccidia in partridges and pheasants in Denmark. *J. Parasitol.* 27: 29-34.
- MÖNNIG, H. O. 1938 Veterinary helminthology and entomology. Wm. Wood and Co., Baltimore. pp. 234-235.
- MOLIN, R. 1858 Prospectus helminthum, quae in prodromo faunae helminthologicae Venetiae continentur. *Sitzungsb. Akad. Wissensch. Wien., Math. Nat. Cl.* 30: 127-158.
- 1860 Una monographia del genero *Dispharagus*. *Sitzungsb. Akad. Wissensch. Wien., Math. Nat. Cl.* 39: 479-516.
- MOORE, E. J. 1933 The parasite problem in game birds. *Atlantic Sportsman* 2: 41.
- NEUMANN, L. G. 1892 A treatise on the parasites. pp. 374-375.
- NEVEU-LEMAIRE, M. 1912 Parasitologie des animaux domestiques. Paris. pp. 828, 831.
- PIANA, G. P. 1897 Osservazioni sul *Dispharagus nasutus* Rud. dei polli e sulle larve nematelmintiche delle mosche e dei porellioni. *Atti Soc. Ital. Sc. Nat., Milano* 36: 239-262.
- PURVIS, M. 1913 (Article not found; listed by Stiles and Hassall, 1920.)
- RABIEAUX, A. 1900 Gastrite épizootique parasitaire de la poule. *J. Med. Vét. et Zootech Lyon* 51: 16-20.
- RAILLIET, A., HENRY A. AND SISOFF, P. 1912 Sur les affinités des dispharages (*Acuaria* Bremser), nématodes parasites des oiseaux. *Compt. Rend. Soc. Biol.* 73: 622-624.
- RANSOM, B. H. 1916 (List of parasites from the Island of Guam.) *J. Parasitol.* 2: 93-94.
- RUDOLPHI, C. A. 1819 Entozoorum synopsis cui accedunt, mantissa duplex et indices locupletissimi. Berolini. pp. 23, 238.
- SEURAT, L. G. 1916 Dispharages d'Algérie. *Compt. Rend. Soc. Biol.* 79: 934-938.
- SKRJABIN, K. I. 1916 Nématodes des oiseaux du Turkestan russe. *Ann. Mus. Zool. Acad. Imper. Sc., Petrograd* 20: 457-557.
- STILES, C. W. AND HASSALL, A. 1920 Index-Catalogue of Medical and veterinary zoology. (Roundworms). U. S. Pub. Health Serv. Hyg. Lab. Bull. 114: 348-349, 691.
- STOSSICH, M. 1891 Il genere *Dispharagus* Dujardin. Lavoro monographico. *Boll. Soc. Adriat. Sc. Nat., Trieste* 13: 81-108.
- VENARD, C. 1933 Helminths and coccidia from Ohio bob-white. *J. Parasitol.* 19: 205-208.
- WALTON, A. C. 1923 Some new and little known nematodes. *J. Parasitol.* 10: 59-70.
- WEBSTER, J. D. 1943 Helminths from the robin, with the description of a new nematode, *Porrocaecum brevispiculum*. *J. Parasitol.* 29: 161-163.
- WEHR, E. E. 1943 Nematodes of poultry. In "Diseases of Poultry," edited by H. E. Biester and L. Devries. pp. 637-677.
- YEATER, R. E. 1934 The Hungarian partridge in the Great Lakes region. *Univ. Mich. Sch. Forestry and Cons. Bull.* 5: 74.
- YORKE, W. AND MAPLESTONE, P. A. 1926 The nematode parasites of vertebrates. J. & A. Churchill, London. pp. 327-331.

## THE ACANTHOCEPHALAN GENUS *CORYNOSOMA*. I. THE SPECIES FOUND IN WATER BIRDS OF NORTH AMERICA

HARLEY J. VAN CLEAVE

University of Illinois, Urbana

Individuals of the acanthocephalan genus *Corynosoma* reach sexual maturity in the digestive tract of birds and mammals, especially those in aquatic habitats or those feeding on fish or aquatic invertebrates which serve *Corynosoma* as intermediate hosts. Although the same species has often been reported from both aquatic birds and mammals, Lundström (1941) has recently reviewed the evidences which seem to discount the possibility that both of these host groups serve as normal host to the same species of *Corynosoma*. In observations on the two best known representatives of the genus in Europe, *Corynosoma strumosum* and *C. sermerme*, Lundström's results support those earlier announced by Forssell (1904, 1905). Both of these species are normal parasites in the intestine of fish-eating mammals, especially seals. Although both species likewise occur often in the intestine of water birds they never attain full functional maturity in these hosts. Larvae of these two species, in the bodies of fish, may be introduced into the digestive tract of birds that prey upon fish but the worms fail to prosper there sufficiently to bring their eggs to full maturity. Evidence of this sort constitutes one of the most valid indictments of the meaningless expansions of "host lists" which received so much attention on the part of parasitologists, particularly a generation ago.

An analysis has been made of numerous collections of *Corynosoma* from various parts of North America. On this continent there are some species which normally parasitize birds and other species which infect mammals. There are no clearly marked evidences of infections occurring in the alternative host group such as have been found for the European species. In the present paper, only those species which infect water birds of North America will be considered. One of these is a well known species, *Corynosoma constrictum* Van Cleave, 1918; the other is a new species here described as *Corynosoma anatarium* n. sp.

Other taxonomic and morphological studies on *Corynosoma* are in the course of preparation for publication.

In 1918, the writer described and named *Corynosoma constrictum*, the first valid species of the genus to be recorded from North American water birds. The material upon which the description was based had been collected by Dr. Edwin Linton from the intestine of the American scoter (*Oidemia americana* Swainson) on Yellowstone Lake in Wyoming. Dr. Linton (1892) had mistakenly identified the worms as "*Echinorhynchus striatus* Goeze" a species described from European water birds. The specific identity and generic assignment of this European species were long under discussion (Lühe, 1911) although Meyer (1931, 1932-3) more recently ascribed it to the genus *Polymorphus*. As pointed out by the present writer in 1918, the males of Linton's original specimens had conspicuous cuticular spines on the fore body (Fig. 10) and around the posterior extremity in the region of the genital pore. This last statement alone is proof of the fact that the individuals are of the genus *Corynosoma*. The genital spines were shown clearly in Linton's

Received for publication, June 25, 1945.



drawings (1892, figs. 18-20) and were likewise recognized by the present writer when he reexamined the original material (see Fig. 9).

Since 1918, the writer has examined many hundreds of specimens of *Corynosoma constrictum* which had been submitted for identification by numerous colleagues. As a result of these studies, *C. constrictum* is now known from at least 10 different host species and from 11 states in addition to records from British Columbia.

Until the life history of this parasite is established, the ecological and economic importance in any given habitat cannot be determined. In many of the localities here recorded, the ducks were killed during their migration. It is consequently uncertain whether their parasites have become established in the region where the infected birds were taken. It is thus possible that *C. constrictum* might be only a seasonal migratory intrusion into the habitats frequented by its migratory hosts. There is no evidence available for establishing any regions as foci for infection by *C. constrictum* and such evidence will in all probability continue to be lacking until the life history of the species becomes known. Broad geographical distribution of this parasite might be dependent upon very extensive geographical distribution of infected intermediate hosts or on the contrary it might be due to wide migratory dispersal of the definitive bird hosts from a very restricted area in their winter habitat where they are exposed to infected intermediate hosts.

Dispersal of parasites in the bodies of migratory birds, even though the infection might have been acquired on another continent, offers opportunity for ultimate extension of geographical range of the parasite. In the numerous localities visited by the infected migratory birds, the opportunity for embryos of the parasitic worms to become established in local arthropods is always present. By this means, parasites transported from a distance by migratory water fowl become potential threats to the native birds and to domestic water fowl.

The following table shows the geographical distribution and the hosts of *Corynosoma constrictum* in the collections of the writer.

Host*	Locality	Collector
"Domestic duck" .....	Illinois	N. D. Levine
	Illinois	E. Bushing
"Teal" .....	New Jersey	F. R. Beaudette
	North Dakota	H. B. Ward
<i>Anas platyrhynchos platyrhynchos</i> (mallard) ....	Illinois	W. C. Starrett
	Iowa	H. J. Griffiths
	British Columbia	C. J. Spencer
<i>Dasila acuta tzitzihua</i> (pintail duck) .....	Illinois	W. C. Starrett
	Wisconsin	W. Olson
	Michigan	W. C. Gower
	British Columbia	C. J. Spencer
<i>Erismatura jamaicensis rubida</i> (ruddy duck) ....	Illinois	W. C. Starrett
	Iowa	H. J. Griffiths
	Ohio	B. Rausch
	Oklahoma	J. E. Guberlet
<i>Fulica americana</i> (American coot) .....	Ohio	B. Rausch
<i>Nettion carolinense</i> (green-winged teal) .....	Illinois	W. C. Starrett
	Texas	E. W. Price
	Iowa	H. J. Griffiths
<i>Nyroca affinis</i> (lesser scaup duck) .....	British Columbia	C. J. Spencer
<i>Nyroca</i> sp? (scaup duck undet.) .....	Iowa	H. J. Griffiths
<i>Oidemia americana</i> (American scoter) .....	Wyoming	E. Linton
<i>Querquedula discors</i> (blue-winged teal) .....	Oklahoma	J. E. Guberlet
	North Dakota	L. B. Dickey
	British Columbia	C. J. Spencer
<i>Spatula clypeata</i> (shoveller) .....	Iowa	H. J. Griffiths

\* Kortright's convenient handbook has been accepted as authority for host names. In some instances, the name that he uses is different from the one accepted by Peters (1931) in his comprehensive catalog of the birds of the world.

In connection with the records shown in the foregoing table, attention should be called to the fact that many of the individual entries of a host from a given locality represent a considerable number of host individuals. In distribution of the sort shown here, where a species utilizes as definitive host various species over a very broad geographical range, the calculations of percentages of infection and records of negative findings are without particular significance and negative records will not be cited. Apparently there is no specificity in host relations for *Corynosoma constrictum*. The chance for any individual of several host species to carry this parasite seems to depend almost wholly upon the availability of food infected with the larvae. The intermediate host of hosts for *C. constrictum* are not known. The diversity in habits, food habits and habitats of the recorded definitive hosts makes it seem probable that some arthropod, particularly a crustacean, is the intermediate host. Food habits of the definitive hosts make it seem doubtful whether fishes serve as reservoir hosts for this species as they do for some of the European members of *Corynosoma*.

It should be noted that in preparing the foregoing table there has been no attempt to determine the synonymy for earlier North American literature records of acanthocephalans in ducks. The obvious misidentification of the original material on which *C. constrictum* is founded, as a European species of an entirely different genus, gives evidence of the futility of any attempt at determining synonymy when the original material is not available.

The writer is deeply indebted to the numerous investigators mentioned in the table for their cooperation in sending material for identification. This is the first of a projected series of articles dealing with the acanthocephalan fauna of North American water birds. The writer expects to complete further studies in the near future giving details of distribution for other species of acanthocephalans of birds. The numerous species of the genus *Polymorphus* are particularly abundant in collections received from these and other collaborators.

The scarcity of records of *Corynosoma* from North American birds in the literature is reflected in the fact that Gower (1939) in his host-parasite catalog of worm parasites of ducks of the world entered only the type host, *Oedemia americana*, as host of *C. constrictum*. At least one other host had escaped Gower's attention since the blue-winged teal (*Querquedula discors*) had been recorded previously as host for this parasite (Van Cleave, 1920). In an earlier paper, Gower (1938) recorded the results of an examination of 104 individuals representing 12 species of ducks, all from Michigan. Although these species included practically all of the species of ducks reported as hosts for *Corynosoma* in the present paper, he gave no record of encountering that genus although 7.7% of the individuals were infested by the acanthocephalan genus *Polymorphus*, which he erroneously called *Filicollis*. The 11 genera of worms for which Gower presented data might not have been all the genera which he encountered, since he made no statement whether he included all forms found or selected only certain groups for comparison. It would at least seem probable that if *Corynosoma* were present in his collections it must have been less well represented than *Polymorphus*.

In 1940, Van Cleave and Starrett listed a total of six species of ducks as host for *C. constrictum*, all from Illinois. All of the earlier records are incorporated in the table here given.

The significance of the genital spines in the taxonomy of *Corynosoma* has been

treated in detail in another paper now in press. In that contribution the writer has demonstrated, for the first time, that a secondary introversion of the hind body in *Corynosoma* may completely conceal the genital spines by withdrawing them, inside the introverted genital vestibule, to a position entirely within the body. No conspicuous instances of genital vestibule formation have been observed for specimens of *C. constrictum*, hence the genital spines are usually readily visible for generic determination, especially in the males. However, the genital spines in females of this species are often very diminutive (Figs. 5, 6) and consequently are not easily seen. This is particularly true when cleared specimens are examined without staining. Furthermore, it is obvious that the genital spines of many female individuals become completely lost (Fig. 7) when the copulatory cap is shed, as first demonstrated by the writer (Van Cleave, 1920). It is pertinent to the present study that *C. constrictum* was the first species of the genus *Corynosoma* to provide the evidence that mutilation is responsible for the loss of genital spines of the female. All earlier investigators had assumed that the lack of these spines was normal and represented the basis for an oft cited instance of sexual dimorphism in *Corynosoma*.

The importance attached to the removal of genital spines of the female has led the writer to make a special study of the structures involved. There is great individual variability in the size of the genital spines on *C. constrictum* even when the individuals are present in the same host specimen. Figs. 4 to 7 show the genital extremity of four different individuals of *C. constrictum* all from the same specimen of the ruddy duck taken on Buckeye Lake in Ohio by Mr. Robert Rausch. On one of the individuals carrying fully formed embryos no spines were observable (Fig. 7) while on another (Fig. 6) two very minute spines could be seen. In the young individuals the spines are never in strict order of arrangement but more than 40 are observable as irregularly dispersed over each lateral surface of the genital tip (Fig. 4, a male).

A number of females have been observed (Figs. 6, 7) in which the area adjacent to the genital pore is apparently moulded into a definite globular projection by the muscular copulatory bursa of the male during copulation. The term genital papilla is applied to this structure.

In males of *C. constrictum*, an abundance of unusually well preserved material has offered a series of stages (Figs. 1-3) for the study of the male copulatory bursa rarely seen in any other species of the ACANTHOCEPHALA. In as much as the copulatory cap is an internal cast of the copulatory bursa, the cement glands and ducts associated with the bursa should all be studied together.

In one cotype individual, a freak of microtechnic resulted in complete opacity of the reservoir of the cement glands with its main duct on one side leading into the bursa and a series of radiating ducts around the periphery of the introverted bursa (Fig. 1). The other individuals of the same species had the bursa extruded in varying degrees and orientation (Figs. 2 and 3). In one of these (Fig. 2), the radiating ducts around the margin of the bursa show their position in the partially extruded bursa. The two muscular pouches (by some called Saeftigen's pouch) which surround the cirrus are shown still within the posterior end of the body. In another specimen (Fig. 3), the fully extruded bursa is shown in rather unusual orientation, in end view with the two muscular pouches lateral to the cirrus.

In the course of critical examination of all of the specimens of *Corynosoma* from

water birds in the collection of the writer, one collection of a species distinct from *C. constrictum* was encountered. This is here recognized as a new species and is described under the name *Corynosoma anatarium*.

*Corynosoma anatarium*, n. sp.  
(Figs. 15-17)

With the characteristics of the genus *Corynosoma*. Body relatively small, lacking conspicuous enlargement of the spine-covered fore body. Genital extremity (Fig. 17) in both sexes provided with a small number of cuticular spines, irregularly scattered. Body length 4.2 to 8.6 mm, maximum diameter 0.9 to 1.7 mm. Proboscis (Figs. 15, 16) about 0.55 mm long and 0.28 to 0.29 mm in maximum diameter; armed with 14 longitudinal rows of 8 or 9 hooks each. Largest hooks approximately 0.088 mm long and 0.023 mm thick at the bend; those near the tip and base somewhat more slender; basal hooks 0.047 to 0.059 mm long and frequently not more than 0.01 mm in thickness; near the tip, 0.059 to 0.082 mm. Hard shelled embryos within body of gravid females 0.1 to 0.112 mm long and 0.02 to 0.023 mm wide, with a short, rounded polar bulge on the second membrane at each pole.

Type host: "Duck" at College Station, Texas, collected by Dr. E. W. Price.

Holotype female (VC 2131.5, Fig. 15) and 11 paratypes (all females except one male, allotype VC 2131.11, with proboscis completely introverted) in collection of H. J. Van Cleave, Urbana, Illinois.

Like *C. constrictum*, the only other species of the genus so far reported from birds of North America, the life history and intermediate hosts are wholly unknown. The two species from North American water birds are most readily distinguishable from each other on the basis of number and size of the proboscis hooks. *C. constrictum* (Figs. 11-14) has from 2 to 4 more longitudinal rows than are found in *C. anatarium* and the largest hooks in the former rarely exceed 0.047 mm in length while in the new species they are commonly 0.088 mm long. A review of all of the species of the genus seems to give evidence of rather definite differentiation of the species of the two hemispheres and fairly sharp cleavage between the normal parasites of birds and those of mammals. However, on morphological basis the new species is rather readily distinguishable from those of other regions of the earth.

*C. anatarium* belongs to the group of species having distinctly less than 18 longitudinal rows of proboscis hooks. Besides *C. constrictum* and *C. anatarium* this group includes the following species: *C. tunitae* reported from water birds of Africa; *C. bullosum* from marine mammals near the antarctic; *C. turbidum* from water birds of the African continent; *C. mergi* from a duck of marine habitats in Sweden; *C. phalacrocoracis* from a cormorant of Japan; *C. peposacae* from water birds of South America; and *C. pyriforme* a practically unknown species from European birds. Of the foregoing *C. turbidum* is readily distinguishable from all others by the one or a few greatly enlarged hooks on the ventral side of the proboscis. *C. tunitae* has a greatly inflated fore body which is lacking in *C. anatarium* and has a genital vestibule concealing genital spines if such are present. *C. mergi* lacks genital spines in the male and these are present in *C. anatarium*. The longitudinal rows of proboscis hooks are more numerous in *C. pyriforme* (16-18 vs. 14 in *C. anatarium*) and in *C. phalacrocoracis* (17 vs. 14 in *C. anatarium*) and the largest hooks in the latter are considerably larger than in the new species. *C. bullosum* and *C. peposacae* have distinctly more hooks on the proboscis and in the latter they are smaller than in *C. anatarium*.

From 1904, when the genus *Corynosoma* was first named and delimited, until 1918 this genus remained unrecognized in the birds of North America. The



description of *C. constrictum* from a single host species of an isolated locality in 1918 has been supplemented in the present paper by additions of other hosts and localities until after a quarter of a century the species is now recognizable as showing no necessary restrictions within the fresh water habitats of this continent and no sharply defined host limitations among the ducks inhabiting our fresh waters. There is at least the possibility that the new species here recognized as *C. anatarium*, with a single host species from a single locality, may likewise become more widely known as larger and more representative collections are studied.

## REFERENCES

- FORSSELL, A. L. 1904 *Echinorhynchus semermis* n. sp. Medd. Soc. Fauna et Flora Fennica 30: 175-179.  
 ——— 1905 Bidrag till Kännedom om Echinorhyncherna i Finlands fiskar. Acta Soc. Fauna et Flora Fennica 27(3): 1-30.  
 GOWER, W. C. 1938 Seasonal abundance of some parasites of wild ducks. J. Wildlife Manag. 2(4): 223-232.  
 ——— 1939 Host-parasite catalogue of the helminths of ducks. Am. Midl. Nat. 22(3): 580-628.  
 KORTRIGHT, F. H. 1942 The ducks, geese and swans of North America. Am. Wildlife Inst., Washington, D. C.  
 LINTON, E. 1892. Notes on avian Entozoa. Proc. U. S. Natl. Mus. 20: 87-113  
 LÜHE, M. 1911 Acanthocephala. Brauer's Süßwasserfauna Deutschlands, Heft 16. Jena.  
 LUNDSTRÖM, A. 1941 Die Acanthocephalen Schwedens mit Ausnahme der Fisch-acanthocephalen von Süßwasserstandorten. Lund. 238 pp.  
 MEYER, A. 1931 Die Acanthocephalen des arktischen Gebietes. Fauna Arctica, Jena 6(1): 1-20.  
 ——— 1932-3. Acanthocephala. Bronn's Klassen und Ordnungen des Tierreichs. Akad. Verlagsgesellsch. M. B. H. Leipzig. 582 pp.  
 PETERS, J. L. 1931 Check-list of the birds of the world, Vol. I. Harvard Univ. Press, Cambridge.  
 VAN CLEAVE, H. J. 1918 The Acanthocephala of North America birds. Tr. Am. Micr. Soc. 37(1): 19-48.  
 ——— 1920 Sexual dimorphism in the Acanthocephala. Trans. Illinois St. Acad. Sc. 13: 280-292.  
 ——— (in press) The genital vestibule and its significance in the morphology and taxonomy of the Acanthocephala, with particular reference to the genus *Corynosoma*. J. Morphol.  
 ——— AND STARRETT, W. C. 1940 The Acanthocephala of wild ducks in central Illinois, with descriptions of two new species. Tr. Am. Micr. Soc. 59(3): 348-353.

## EXPLANATION OF PLATES 1 AND 2

All drawings were made with the camera lucida from stained individuals mounted in Balsam or Damar. Katharine Hill Paul, scientific artist in the Department of Zoology of the University of Illinois, made all of the illustrations.

- bursa—male copulatory bursa
- cement ducts—meridional cement ducts in bursa
- cement gl.—cement glands of male system
- cement res.—reservoir of cement glands
- gen. pap.—genital papilla of female
- gen. pore—genital pore of female
- gen. spines—genital spines, both sexes
- lem.—lemnisci
- musc. pouches—muscular pouches of male copulatory bursa
- testes—male gonads
- x—position of detail shown in Fig. 10.

## PLATE 1

Details in the morphology of *Corynosoma constrictum* Van Cleave, 1918

Each entire line accompanying Figs. 1-3 has the value of 0.5 mm, all other figures on this plate are at uniform magnification with scales having value of 0.1 mm.

## PLATE 1

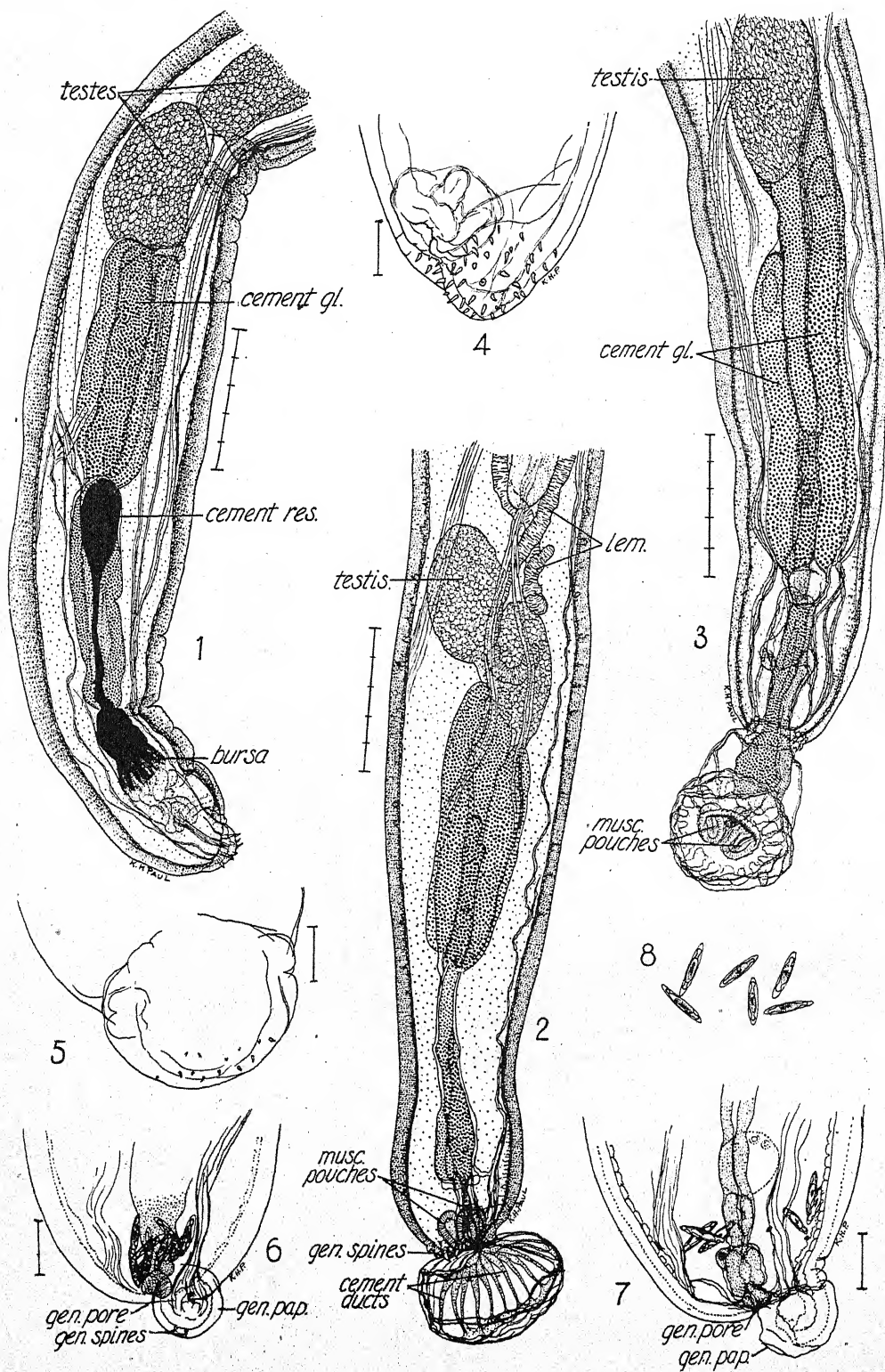


PLATE 2

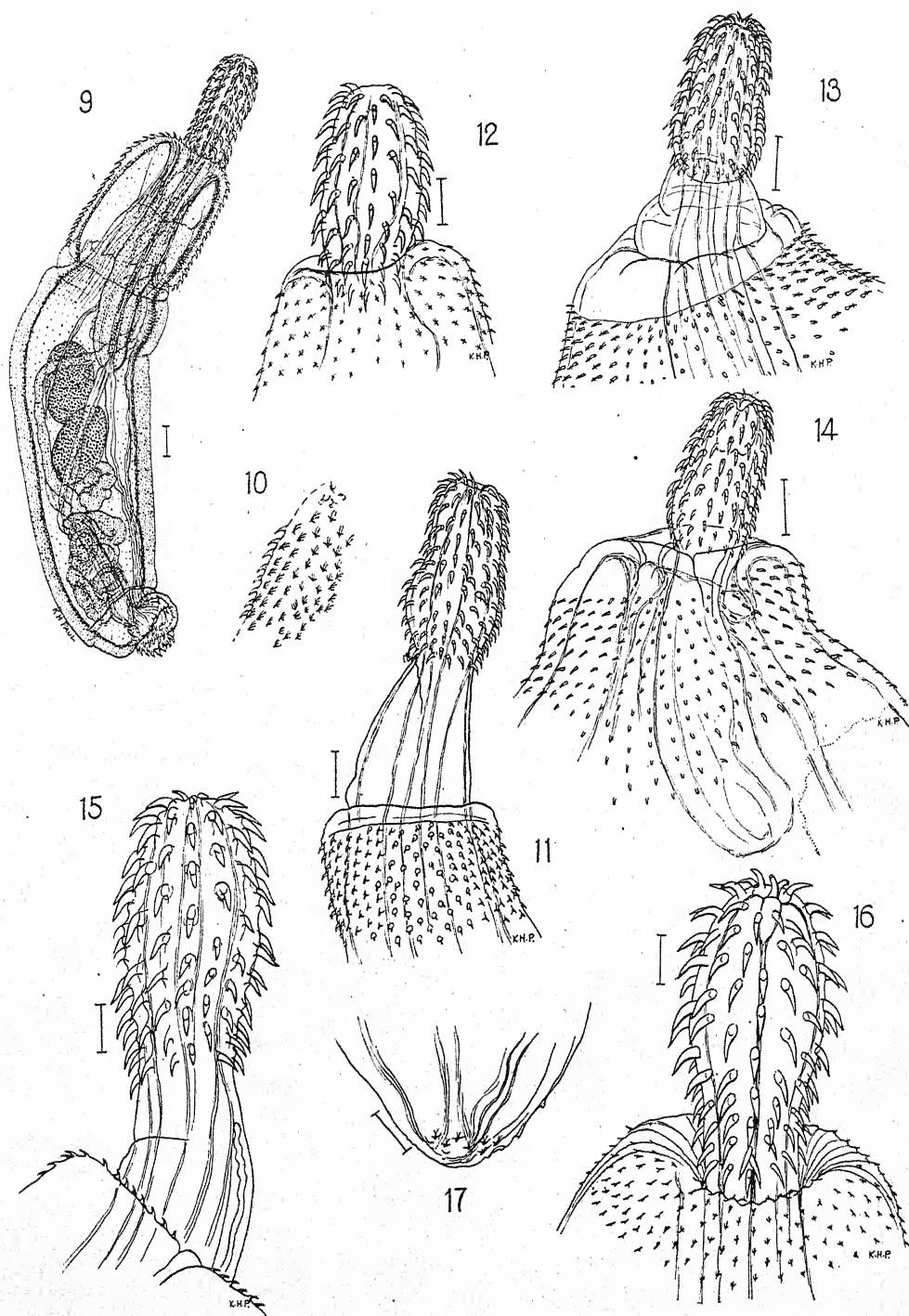


FIG. 1. Posterior end of a cotype male in which the cement reservoir and its ducts became opaque. Of the two main ducts, only the one on the right side of the body is shown. The digitiform ducts are within the margin of the bell of the introverted copulatory bursa.

FIG. 2. Posterior end of a male with copulatory bursa almost wholly extruded but with muscular pouches still within the body. Note the ducts from the cement reservoir running meridionally in the extruded bell of the bursa. Position of genital spines, anterior to the front margin of the extruded bursa, is shown.

FIG. 3. Copulatory bursa completely extroverted, in ventral view showing openings of the meridional cement ducts and the two muscular pouches lateral to the small median cirrus.

FIGS. 4-7. Posterior ends of a series of individuals from a ruddy duck (Ohio) to show sexual and individual variations in size, number and arrangement of the genital spines.

FIG. 4. Genital spines of a male.

FIG. 5. Small, scattered genital spines of a female.

FIG. 6. A female from which all but two of the genital spines had been lost from the genital papilla.

FIG. 7. A female showing no genital spines on the genital papilla.

FIG. 8. Several mature embryos from the body cavities of two different females from the ruddy duck.

#### PLATE 2

Details of the morphology of *Corynosoma constrictum* Van Cleave, 1918 and *C. anatarium* n. sp.

On this plate the line beside each drawing indicates the value of 0.1 mm.

FIG. 9. A cotype of *C. constrictum* showing details of morphology. Note that body spines are omitted from the lateral surface of the fore body (their arrangement at "x" shown in Fig. 10).

FIG. 10. Detail showing shape and pattern of arrangement of spines of the fore body at region in Fig. 9 marked "x."

FIGS. 11-14. Variations in appearance of proboscis, neck, and fore body in *Corynosoma constrictum*.

FIG. 11. Proboscis, neck, and fore body fully extruded.

FIG. 12. Neck fully telescoped within fore body.

FIGS. 13 and 14. States of retraction of neck into fore body.

FIGS. 15-17. *Corynosoma anatarium* n. sp.

FIG. 15. Proboscis, neck and fore body, the latter with spine field shown in optical section, omitting the spines on the lateral surface of the body.

FIG. 16. Individual with neck entirely telescoped within fore body, the latter showing arrangement of body spines.

FIG. 17. Posterior extremity of a gravid female showing many of the genital spines intact.



## A SCRUB TYPHUS (TSUTSUGAMUSHI) OUTBREAK IN DUTCH NEW GUINEA

JAMES T. GRIFFITHS, JR., CAPT. SN. C.

Little information concerning scrub typhus (tsutsugamushi fever) as it occurs in Dutch New Guinea has ever been recorded. Gunther (1940) stated that although numerous cases of scrub typhus had been noted in territories under Australian mandate, no cases had ever been reported from Dutch New Guinea. It is known, Kohls et al (1945), that in New Guinea the disease is caused by *Rickettsia orientalis*, that these organisms are found in native wild rat populations, that certain species of mites belonging to the subfamily TROMBICULINAE are vectors, and that these mites appear to prefer grassy moist areas in which to complete their development. This report is a study of an epidemic of scrub typhus which occurred among American troops during August and September of 1944 at an advanced base in Dutch New Guinea.

On 30 July 1944, elements of a United States infantry division with attached units landed on the 20-mile stretch of enemy-held coastline between Cape Sansapor and Cape Opmarai on the Vogelkop peninsula of Dutch New Guinea. The first typhus casualty became ill on 6 August. Subsequently through 30 September there were 931 cases and 34 deaths. Fig. 1 shows the number of hospital admissions per day for all troops on the base. Since daily admissions fluctuated considerably, the line showing seven-day averages presents a better history of the casualty rate. It will be noted that the outbreak reached epidemic proportions almost immediately, but that after two weeks the rate gradually decreased. By 30 September there was about one typhus casualty being admitted to the hospital per day.

### RELATIONSHIP BETWEEN TYPHUS INCIDENCE AND ENVIRONMENT

Figs. 2 and 3 show the original terrain features as they existed prior to the landings. Although troops were present on both Middleburg and Amsterdam Islands, the majority of the camp installations was found between the Table and Yuma Rivers on the mainland. Additional outposts were maintained for a time at the Kor River at Cape Opmarai, and at Sansapor village. By questioning individual patients and by studying the movements of organizations on the base, it was possible to determine the geographical source for most of the typhus cases, and thus, definite relationships between the various environments and typhus incidence could be made. Four types of environments were readily distinguishable. These included abandoned native village and garden areas, overgrown coconut groves, vine-grass associations at the forest and beach margins, and climax rain forest. Each is discussed below.

*Abandoned native village and garden areas.*—Scattered along the entire coast were semi-cleared areas which appeared to have been either native villages, small plantation areas, or the sites of former native gardens. Coconut, banana, papaya, and kapok trees were often present, but probably exerted little or no effect upon

the ecology of the typhus mite. Grass had established itself throughout these areas. It was knee high and rank enough that the soil was shaded and remained moist except during dry weather.

More than 500 cases of scrub typhus were contracted in this type of environment. The most important typhus sources (see Figs. 2 and 3) were Mar Village (300 cases), Sansapor village (175 cases), and cleared areas in the vicinity of the Kor River and Cape Opmarai (75 cases). Although small numbers of Japanese soldiers had lived in some of these localities, there is no evidence to support a theory that their presence was in any way connected with the typhus incidence of these local areas. This environmental type definitely produced a higher incidence among the personnel involved than any other type of habitat.

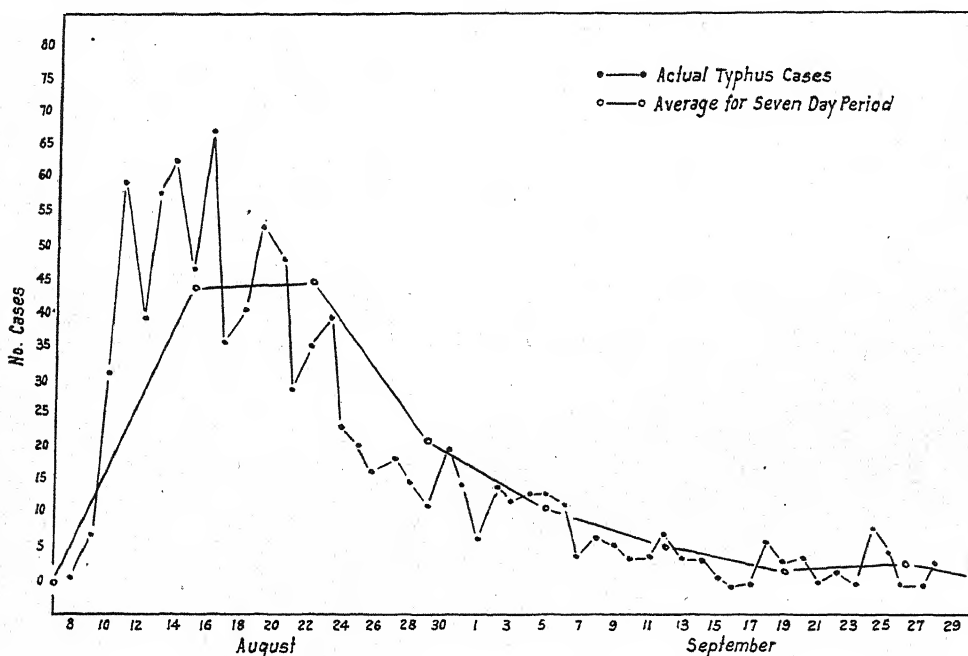


FIG. 1. Scrub typhus hospital admissions per day.

*Overgrown coconut groves.*—Middleburg Island was three and Amsterdam Island six miles from the mainland. Both were covered with coconut groves which had been neglected during the Japanese occupation. A dense undergrowth had arisen between the trees. This was composed largely of young coconut trees and ferns, but small amounts of grass and scrubby growth were also present. Although there was sufficient shade to maintain a moist topsoil, a low typhus incidence (21 cases on Middleburg and five cases on Amsterdam) occurred. No satisfactory explanation for this low rate can be offered at this time, but it is suggested that the lack of grass may possibly have contributed to a reduced mite population. No extensive areas of this type were present on the mainland although a small coconut grove was present at Sansapor village.

*Beach and forest margins.*—A strip of grass and ground-clinging vines extended along the entire beach from east of the Kor River to west of Sansapor village.

This floral association varied in width from about five to 30 yards and was found at the margin between rain forest and sandy beach. Part of it was exposed to direct sunlight during the greater part of the day and moist topsoil was maintained only where shade was furnished by forest margin trees. Although 90 cases of typhus were recorded from this environment, the incidence was low as compared with those in Mar and Sansapor villages.

*Climax rain forest.*—This type of environment covered most of the base. It was composed of tall trees whose interlocking branches produced a dense shade. No grass grew in the forest and the bare ground was covered only by moist rotting

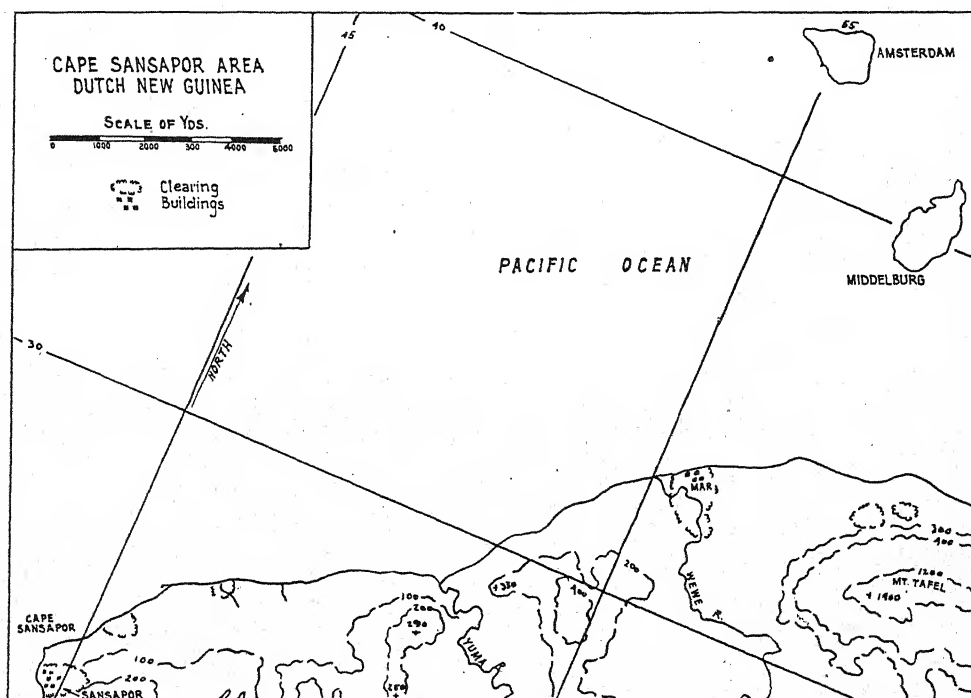


FIG. 2.

leaves. Although it was impossible to definitely trace a single case of typhus to this environment, it should be noted that scrub itch due to the bites of larval mites was a common occurrence among unprotected individuals working in virgin rain forest. In one region a road paralleled the beach and acted more or less as a boundary between the vine-grass association on one side and true rain forest on the other. Although comparable numbers of men lived on both sides of the road, only scrub itch was reported from the rain forest area, while on the beach side of the road 20 typhus cases developed. It appears reasonable to assume that rain forest was probably not a source of scrub typhus.

#### INCUBATION PERIOD

For this epidemic it was possible to determine from certain case histories that the typhus incubation time varied within a range of 7 to 17 days and averaged 11

days. One soldier who landed at Mar village on 30 July became ill on 6 August and was admitted to the hospital three days later. In this case, seven days represented the longest possible incubation time. Maximum periods were first suggested when it was noted that an organization usually became typhus free within 16 days after moving to a non-infectious area.

However, the most information on incubation times was obtained when the 2nd Battalion of one infantry regiment went on patrol to the Kor River. The battalion departed for the Kor River on 10 August. That night they slept on the ground in a cleared area about 500 yards west of the river. The next morning they moved up to the river and encamped on both sides of the stream. On the morning of 12

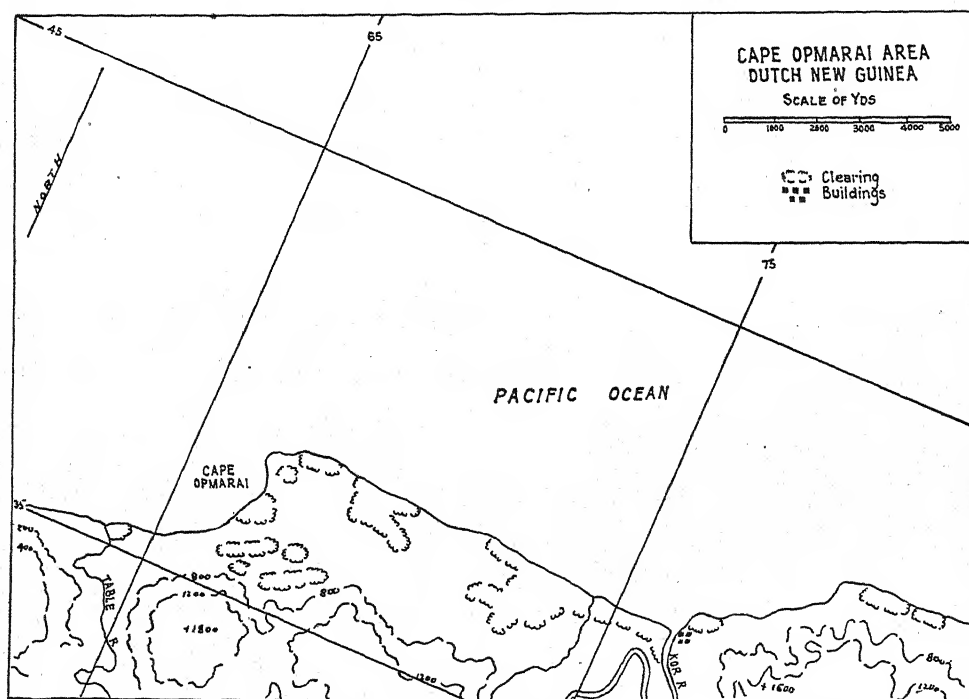


FIG. 3.

August, the entire group returned to its original uninfected camp site near the Table River. Since troops were on the march during daylight hours of 10 and 12 August, it seems reasonable to assume that the majority of the 44 typhus cases were contracted during the 36-hour period between 6 PM on 10 August and 6 AM on 12 August. Fig. 4 illustrates the daily admission rates for this series of cases. It was determined by interviews with more than 100 patients that initial symptoms were generally developed two days prior to hospitalization. With this fact in mind, the minimum possible incubation time for this group was seven days and the maximum possible time was 17 days. If it is assumed that 11 August was the date of exposure and that the onset of symptoms occurred two days prior to hospitalization, the average incubation period becomes approximately 11 days.



## FACTORS INFLUENCING TYPHUS CONTROL

*Clearing unit areas.*—At the Sansapor-Opmarai base the removal of all grass and undergrowth from an infested area proved to be a practical means of typhus control. When the top soil had sufficient time to thoroughly dry out, it appeared that the area could be considered as typhus-free. In numerous instances troops unknowingly moved into infested areas. Those organizations which cleared their camp sites immediately had only a few initial cases. On the other hand, those units which failed to properly clear such infested areas suffered typhus casualties over prolonged intervals of time.

*Rat control.*—Since in New Guinea rats are the known animal reservoir for the rickettsiae of scrub typhus, rat control as a long range program would appear to

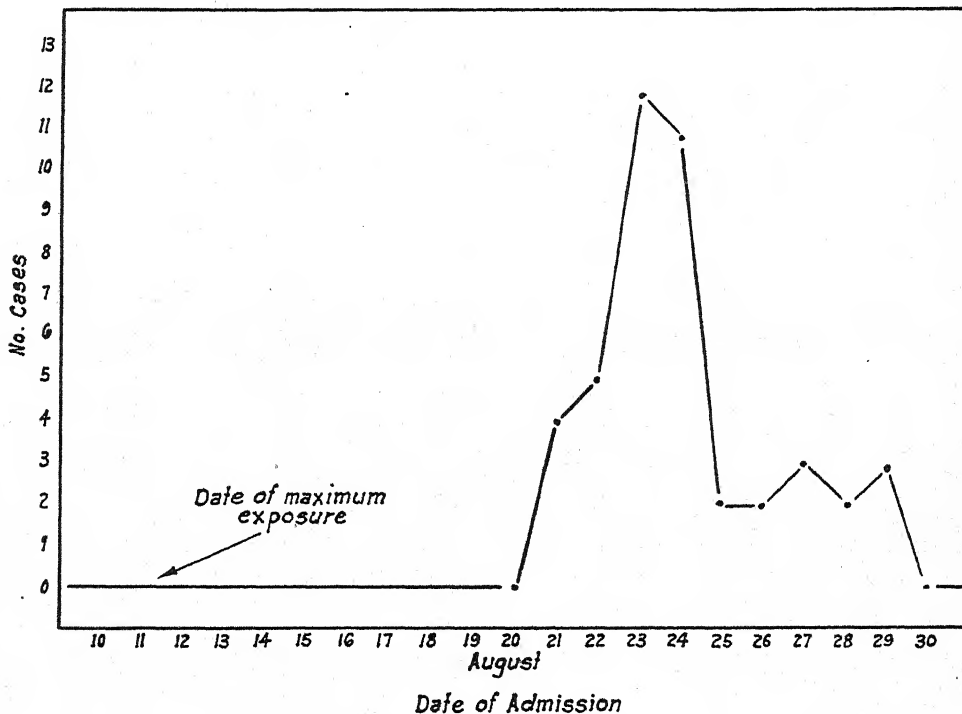


FIG. 4. Typhus incidence in 2nd Battalion.

be desirable. Although a rat control program was instituted early in this epidemic, no evidence as to its beneficial effect could be demonstrated. Under conditions prevailing in New Guinea where adjacent uninhabited areas contain large rat populations, it appears that during short-term military operations rat control does not offer a practical means for controlling scrub typhus.

*Clothing treatment.*—Although clearing an area represents a means of long-term control, under combat conditions protection must be obtained for those men actually engaged in combat or on patrol. Knipling and Dove (1944) state, "The most effective means of protection from chiggers (Trombiculid mites) was found to be the application of liquid repellents (dimethyl phthalate, Indalone, Rutgers 612) to clothing. Clothing can be sprayed with repellent or dipped in repellent solutions

with effective results." The modified clothing treatment developed by Captain R. C. Bushland, Sn.C., of the United States of America Typhus Commission was used after the diagnosis of scrub typhus had been made at Sansapor. This method is the impregnation of clothing with dimethyl phthalate-soap emulsion. On both Middleburg Island and the mainland, some clothing was treated as early as 4 August. However, this involved small units and cannot be considered as being instrumental in affecting the over-all typhus picture. It was not until 1 September, after supplies became available, that most of the troops were fairly consistently wearing treated clothing, and by that time between 850 and 900 cases of typhus had already been contracted. Even if clothing treatment had been generally instituted by 15 August, because of lag incubation (see Fig. 1), more than 700 casualties would have occurred. The protective value of clothing treatment, where properly used, was well demonstrated by several units. These instances are described below.

By comparing the typhus rates of the three rifle companies (I, K and L) of the 3rd Battalion of one infantry regiment, it was possible to demonstrate the beneficial effects of clothing treatment. On 31 July these companies had arrived at Sansapor village. Although Company K remained in the highly infectious Sansapor area, I and L Companies on 11 August moved to an area of low typhus incidence at the mouth of the Yuma river (see Fig. 2). Therefore, other factors being equal, it was to be expected that the admission rate for I and L Companies would fall below that for K Company. As shown in Figure 5, the exact opposite was true. At this point it should be noted that Company K had begun systematic clothing treatment on 14 August, but the other two companies did not begin treatment until more than two weeks later. As indicated on the graph, zero day represents the date of initial clothing treatment for K Company and the date of movement from Sansapor for I and L Companies. If clothing treatment were effective, typhus admissions from K Company should be reduced after zero plus 19 days. As indicated on the graph, this was exactly what happened. In contrast to the one casualty in K Company, I and L suffered 11 cases during a comparable number of days. This was in spite of the fact that K Company remained in the highly infectious Sansapor area.

It may be argued that the clearing of the Sansapor area rather than clothing impregnation was responsible for the reduction in K Company's typhus rate. However, two signal air warning units and one anti-aircraft organization had remained with K Company at Sansapor. They did not treat their clothing and they had a steady typhus rate throughout August and early September. In view of this, the conclusion appears justified that clothing treatment was the important factor in reducing the typhus incidence in K Company.

One section of beach which possessed a typical vine-grass association was occupied by seven different units on about 13 August. Table 1 lists the units in the order in which they lived on the beach, the status of clothing impregnation, the number of men in each, and the number of typhus cases. The marked contrast in typhus incidence between units wearing properly treated clothing and those not wearing such clothing is well demonstrated in this table. The organizations wearing properly treated clothing had no typhus cases as opposed to 15 per cent typhus casualties for those units without impregnated clothing. It appeared that clothing impregnation was the factor which produced this difference in the number of typhus cases.

One infantry regiment arrived at the Sansapor-Opmarai base on 23 August, 25 days after the initial landing. All men were ordered to wear treated clothing when they disembarked. The regiment moved into a beach and rain forest area

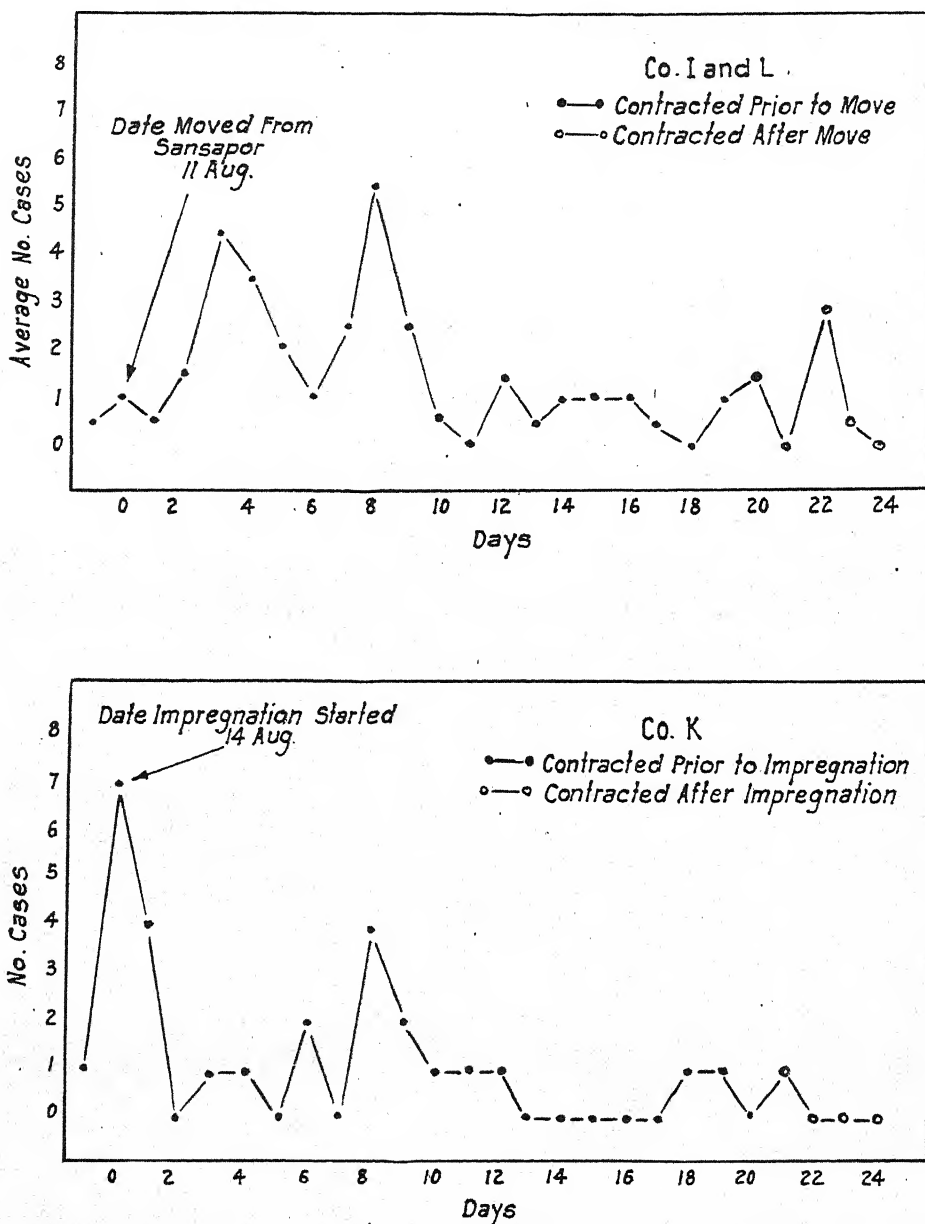


FIG. 5. Typhus cases from rifle companies of 3rd Battalion.

where it reported only two cases of typhus. These were contracted during the first week at the base and both admitted their failure to wear treated clothing during that period. Thus, an entire regiment had only two cases out of approximately 3000 men. This compares very favorably with a field artillery battalion in a similar

and adjacent area which wore no treated clothing when moving into its area and which had an incidence of three cases for 800 men.

As previously stated, the 2nd Battalion of one infantry regiment was on a patrol at the Kor River and during a three-day exposure period contracted 44 cases of typhus among about 800 men. Company I of the same regiment followed the 2nd Battalion, remained one week at the river, and contracted 16 cases among approximately 180 men. Company L replaced I Company and was in the area for seven days. However, its members wore treated clothing and they suffered only four typhus casualties. All three units engaged in similar activities and camped on essentially the same site while at the river. When they are compared on the basis of cases per man days of exposure, the rates for the 2nd Battalion and for I Company are four to five times higher than for L Company. Here again, the unit wearing impregnated clothing had a greatly reduced typhus incidence.

TABLE. 1.—*Typhus cases and status of clothing impregnation for seven units living side by side*

Organization	Status of clothing	No. men	Cases typhus
Unit	Not impregnated	15	3
Unit	Impregnated	12	0
Unit	Impregnated	13	0
Unit	Not impregnated	100	8
Unit	Not impregnated	35	12
Unit	Impregnated	36	0
Unit	Impregnated	12	0

All of the 126 men admitted to the hospital during the month of September had some opportunity to have learned about treated clothing and to have worn it. Of this group 67, or more than 50 per cent, were interviewed by the author. Only one man of the 67 had faithfully worn impregnated clothing (trousers, shirt, and socks) throughout the time of possible exposure. He gave a daily history of walking naked through 25 yards of vines and grass when going to and from ocean bathing. However, even if his case was a failure for clothing treatment, only one man in 67 represents a very low percentage.

Clothing treatment cannot be expected to result in 100 per cent control since sleeping on the ground or in fox holes, failure to wear impregnated socks, and the improper treatment of clothing will allow some break-throughs. However, on the basis of the above facts, it may be concluded that treatment will markedly decrease the number of typhus cases in infectious areas.

#### MEDICAL STATISTICS

In order to review the medical aspects of the situation, a letter written to the Division Surgeon by Major Robert W. Hollenhorst, M.C., is quoted. It reviews a sample of 275 cases and is in complete agreement with the findings of the other units that hospitalized typhus cases at the Sansapor-Opmarai base.

The typical initial cutaneous lesion, the eschar, was present in 92 per cent of cases, with swelling and tenderness of the local lymph glands in all cases with the eschar. The eschars were situated as follows: 24 per cent in the axillary region, 20 per cent on the scrotum, 11 per cent on the thighs, 8 per cent in the inguinal region of the abdomen, 8 per cent on the penis, 6 per cent on the back, 5 per cent on the abdomen, and 4 per cent on the arms above the elbows. There were 4 patients (2 per cent) who had lesions on the eyelids, and two patients with lesions on the neck.

Of the other symptoms manifested by these patients, the fever, general body aches, backache and eye-pain were fairly constant findings. Ninety per cent of patients had an intractable head-



ache, 3 per cent had hiccoughs of severe degree, 11 per cent had troublesome diarrhea, 30 per cent had from moderate to severe constipation, 5 per cent complained of cough, and 45 per cent had marked photophobia. Cough was a fairly frequent complication, as was a transitory deafness. Over three-fourths of these patients complained of moderate to severe insomnia.

At approximately the second day of the disease, 95 per cent of these patients developed a generalized lymphadenopathy. About the third to the sixth day of the disease, a maculo-papular rash, similar to that of measles, but located over the body and extremities, appeared in 28 per cent of these patients. This rash persisted to about the tenth to twelfth day, and seemed to have no relationship to the severity of the disease.

The fever was continuous throughout the disease, oscillating between 101 and 104 degrees daily. This fever ultimately fell by lysis in 70 per cent of the cases, and by crisis in the remainder. It was noted that those in which the fever fell by crisis had a course of much shorter duration, 11 to 16 days, and that the disease on the whole was much more mild; while in those in which the fever fell by lysis, the course of the disease was more severe, from 14 to 21 days, and was followed by a much slower convalescence and moderate to marked anemia.

This particular epidemic was characterized by a high incidence of basal brain involvement in that approximately 1 per cent of cases showed delirium, varying pupillary changes, intractable and exquisite headaches, nystagmus, and increased tendon reflexes. In one case only, stiffness of the neck muscles and positive Kernig's sign was noted. Spinal puncture was done in 12 cases, two of which showed xanthochromic fluid and moderate increase in the number of lymphocytes, but the rest of these cases had a clear fluid with a mild increase in lymphocytes. No case showed increased intraspinal pressure. All cases that died, of which there were eight, died a cerebral death, although there were signs in all cases of terminal collapse of the peripheral circulatory system with cyanosis, respiratory distress, and fall of the blood pressure.

Thirty-four deaths (3.65 per cent mortality) occurred among 931 cases of typhus. Table 2 lists the fatal cases and shows the apparent source of infection, date admitted to the hospital, date of death and (assuming two days illness prior to hospitalization) the duration of the disease prior to death.

TABLE 2.—Typhus mortality statistics

Source of infection	Date of hospitalization	Date of death	Days from onset to death
Undetermined	2 Sept.	11 Sept.	11
Undetermined	2 Sept.	11 Sept.	11
Mar village	13 Aug.	22 Aug.	11
Mar village	11 Aug.	20 Aug.	11
Vine-grass	12 Aug.	22 Aug.	12
Mar village	29 Aug.	8 Sept.	12
Sansapor	30 Aug.	9 Sept.	12
Sansapor	15 Aug.	25 Aug.	12
Sansapor	19 Aug.	30 Aug.	13
Sansapor	16 Aug.	27 Aug.	13
Sansapor	18 Aug.	29 Aug.	13
Vine-grass	5 Sept.	16 Sept.	13
Mar village	11 Aug.	22 Aug.	13
Undetermined	25 Sept.	6 Oct.	13
Sansapor	10 Aug.	22 Aug.	14
Undetermined	11 Aug.	23 Aug.	14
Sansapor	14 Aug.	26 Aug.	14
Sansapor	16 Aug.	28 Aug.	14
Mar village	17 Aug.	30 Aug.	15
Mar village	21 Aug.	3 Sept.	15
Sansapor	16 Aug.	30 Aug.	16
Vine-grass	28 Sept.	12 Oct.	16
Mar village	16 Aug.	31 Aug.	17
Sansapor	16 Aug.	31 Aug.	17
Mar village	10 Aug.	25 Aug.	17
Mar village	10 Aug.	25 Aug.	17
Vine-grass	23 Aug.	8 Sept.	18
Sansapor	20 Aug.	5 Sept.	18
Sansapor	23 Aug.	10 Sept.	20
Mar village	16 Aug.	3 Sept.	20
Sansapor	13 Aug.	31 Aug.	20
Sansapor	23 Aug.	11 Sept.	21
Sansapor	16 Aug.	8 Sept.	25
Mar village	24 Aug.	17 Sept.	26

Reference to Table 2 shows that of the fatal cases 15 contracted the disease at Sansapor, 11 at Mar village, four in a vine-grass association, and four from unknown sources. It is questionable if any conclusion regarding virulence at a given

locality should be made from these data. Death occurred in from 11 to 26 days with an average of 15 days.

#### INCIDENCE AMONG JAPANESE TROOPS

Very little information has been acquired concerning the incidence of scrub typhus among Japanese troops in this area. One Japanese prisoner of war was admitted to the hospital in September. His condition was diagnosed as scrub typhus with initial malaria complication. He died on 25 October with typhus fever listed as the cause of death.

#### SUMMARY

A scrub typhus epidemic among American troops in Dutch New Guinea is described. During a 60-day period there were 931 cases with 34 deaths (3.65 per cent mortality). The various factors concerned in the relationship between environment and typhus incidence are listed and discussed. Effective methods for controlling scrub typhus under jungle conditions are suggested. Medical statistics including incubation times are provided. One case of typhus in a Japanese soldier is reported.

#### CONCLUSIONS

1. For this epidemic, incubation times varied from seven to 17 days and averaged 11 days.
2. Scrub typhus was associated with grassy areas or with locations that contained some type of low dense ground cover.
3. Clearing of unit areas and the wearing of properly treated clothing were effective means for controlling scrub typhus under jungle conditions.

#### REFERENCES

- GUNTHER, CARL E. 1940 A survey of endemic typhus in New Guinea. *Med. J. Australia*, 30 November, pp. 564.
- KOHL, G. M., ARMBRUST, C. A., IRONS, E. N. AND PHILIP, C. B. 1945 Studies on tsutsugamushi disease (scrub typhus, mite-borne typhus) in New Guinea and adjacent islands: further observations on epidemiology and etiology. *Am. J. Hyg.* 41: 374-396.
- KNIPLING, E. F. AND DOVE, W. E. 1944 Recent investigations of insecticides and repellants for the armed forces. *J. Econ. Ent.* 37: 477-489.

AMERICAN SOCIETY OF PARASITOLOGISTS  
PRELIMINARY ANNOUNCEMENT OF THE TWENTIETH ANNUAL MEETING

THURSDAY TO SATURDAY, MARCH 28 TO 30, 1946  
ST. LOUIS, MISSOURI

The American Society of Parasitologists will convene for a three-day program meeting in conjunction with the convention of the American Association for the Advancement of Science in St. Louis, Missouri. The sessions will be held in the Municipal Auditorium.

The program will probably be arranged as in previous years, with all of the sessions of the first and third days devoted to contributed papers. The afternoon session of the second day will be given over to papers presented by demonstration and members are urged whenever possible to present or illustrate their work in this manner since it affords greater opportunity for informal discussion than is possible at other program sessions. Papers which are read may also be presented by demonstration.

The address of the retiring President, Dr. A. C. Chandler, *THE MAKING OF A PARASITOLOGIST*, will be scheduled for 11 AM on the second day of the meeting. This will be followed by the annual luncheon and the general business meeting of the Society. Tea will be served during the afternoon demonstration program.

The call for papers will be sent to all members of the Society. Abstracts of papers to be read in person, or by title, or presented by demonstration must be in the office of the secretary by December 1, 1945. These abstracts will be printed as a supplement to the December, 1945, issue of the *Journal of Parasitology*. Members are hereby informed of the final date for submitting abstracts and are urgently requested, because of current unavoidable difficulties in publication, to be prompt in mailing their abstracts for the program.

Respectfully,

JAMES T. CULBERTSON,  
*Secretary*

# Journal of Parasitology

## Back Numbers

Only a few partial sets in the first 18 volumes still unsold at special prices.

Single copies of some early volumes and many odd numbers available; while the supply lasts at \$7.50 per volume, \$1.50 per number.

*Address*

# Journal of Parasitology

New York University,  
University Heights,  
New York 53, N. Y.

N. Queen St. & McGovern Ave.,  
Lancaster, Pa.



# The Journal of Parasitology

Volume 31

December Supplement, 1945

## PROGRAM AND ABSTRACTS OF THE TWENTIETH ANNUAL MEETING OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

SAINT LOUIS, MISSOURI

MARCH 28, 29, and 30, 1946

### PROGRAM<sup>1</sup>

THURSDAY MORNING SESSION, MARCH 28, 10:00 AM; MUNICIPAL AUDITORIUM.

#### Read

1. Comparison of Methods Used for the Detection of *Endamoeba histolytica*. (7 min) OSCAR FELSENFELD, Mt. Sinai Medical Research Foundation, Chicago.
2. The Staining of Protozoa in Formolized Stool Specimens. (6 min) VIOLA MAE YOUNG, Mt. Sinai Medical Research Foundation, Chicago.
3. The Influence of Whole Egg Inoculum on the Growth of *Endamoeba histolytica*—Organism *t* in Enriched Egg White Medium and in Whole Egg Dialysate Medium. (15 min) (Lantern) CHARLES W. REES, LUCY V. REARDON, ELEANOR M. JOHNSON, AND M. FRANCES MAYFIELD, National Institute of Health.
4. Pathogenicity of Pure Culture *Trichomonas gallinae* Orally Administered to Clean Pigeons. (10 min) ROBERT M. STABLER AND FRANK B. ENGLEY, JR., University of Pennsylvania.
5. Further Studies on the Inoculations of *Trichomonas foetus* (Protozoa) in Heifers. (12 min) (Lantern) BANNER BILL MORGAN, University of Wisconsin.
6. Toxicity of Cecal Cores from Chickens Infected with the Protozoan, *Eimeria tenella*. (10 min) MARY JANE BRADFORD AND C. A. HERRICK, University of Wisconsin.
7. Dosage of *Eimeria stiedae* Related to Severity of Liver Coccidiosis. (12 min) (Lantern) HARRY A. JANKIEWICZ, College of Osteopathic Physicians and Surgeons, Los Angeles.

THURSDAY AFTERNOON SESSION, MARCH 28, 2:00 PM; MUNICIPAL AUDITORIUM.

#### Read

8. Cryptozoites and Metacryptozoites of *Plasmodium relictum* in Canaries and Pigeons. (12 min) (Lantern) FREDERICK COULSTON AND CLAY G. HUFF, University of Chicago.
9. *Plasmodium circumflexum* in the Ruffed Grouse in Ontario. (15 min) (Lantern) A. MURRAY FALLIS, Ontario Research Foundation, Toronto.

<sup>1</sup> An alphabetical author index will be found at the end of the program. Extra copies of this Supplement, and portraits of parasitologists, will be on sale at the meeting.

10. Studies on *Plasmodium gallinaceum* Brumpt. IV. Parasitemia and Survival in Blood-Induced Infections in Young Chicks. (15 min) (Lantern) W. CLARK COOPER AND G. ROBERT COATNEY, National Institute of Health.
11. Studies on *Plasmodium gallinaceum* Brumpt. VI. A Study of the Pathology in Young Chicks. (15 min) (Lantern) LLOYD R. HERSHBERGER AND G. ROBERT COATNEY, National Institute of Health.
12. *In vitro* Studies on Malarial Sporozoites. (15 min) (Lantern) RICHARD J. PORTER, RAYMOND L. LAIRD, AND ELIZABETH DUSSEAU, University of Michigan.
13. Observations on the Courses of Infection in Sporozoite-Induced *Plasmodium cynomolgi* Malaria. (15 min) (Lantern) RAYMOND L. LAIRD AND RICHARD J. PORTER, University of Michigan.
14. *Plasmodium elongatum* in Pekin Ducks. (15 min) (Lantern) FRUMA WOLFSON, Johns Hopkins University.
15. The Relations between Pantothenic Acid and *Plasmodium gallinaceum* Infections of the Chicken. (15 min) (Lantern) STERLING BRACKETT AND EMANUEL WALETZKY, American Cyanamid Company, Stamford Connecticut.
16. The Relative Activity of Sulfanilamides and Other Compounds in *Eimeria tenella* Infections in the Chicken. (15 min) (Lantern) EMANUEL WALETZKY AND CARRIE OLA HUGHES, American Cyanamid Company, Stamford, Connecticut.
17. Aspects in the Control of Intestinal Coccidiosis in Commercial Rabbitries by the Use of Phthalylsulfathiazole. (12 min) (Lantern) EVERETT E. LUND, United States Rabbit Experiment Station, Fontana, California.

*By Title*

18. Chemical Studies on Egg White Medium for the Cultivation of *Endamoeba histolytica*. THEODOR VON BRAND, CHARLES W. REES, LUCY V. REARDON, AND WILLIAM F. SIMPSON, Catholic University of America and National Institute of Health.
19. Hexenolactone, an Effective Inhibitor of *Trypanosoma cruzi* growth *in vitro*, but not *in vivo*. THEODORE S. HAUSCHKA, N. DU BOSE MAXWELL, AND ELEANOR M. JOHNSON, Lankenau Hospital Research Institute and National Institute of Health.
20. Studies on *Plasmodium gallinaceum* Brumpt. V. Quinine Standardization in Screening Tests Against Blood-Induced Infections in Young Chicks. G. ROBERT COATNEY AND W. CLARK COOPER, National Institute of Health.

FRIDAY MORNING SESSION, MARCH 29, 9:00 AM; MUNICIPAL AUDITORIUM.

*Read*

21. The Life History Cycle of *Diectophyma renale*, the Giant Kidney Worm of Man and Many Other Mammals. (15 min) (Lantern) ARTHUR E. WOODHEAD, University of Michigan.
22. Epidemiological Investigations on Filariasis on Certain Islands of the South Pacific Area. (12 min) (Opaque projection) ELON E. BYRD, University of Georgia.
23. Studies in Filariasis. II. A Skin Test for Filariasis *bancrofti* Utilizing Antigen Prepared from Microfilariae of *Wuchereria bancrofti*. (15 min) (Lantern)

GEORGE W. HUNTER, III, JOHN BOZICEVICH, AND VIRGINIA G. WARREN, Army Medical School and National Institute of Health.

24. The Biological Status of *Sarcocystis*. (15 min) L. A. SPINDLER AND HARRY E. ZIMMERMAN, JR., U. S. Bureau of Animal Industry.

25. Studies on Imported Malarias: 4. The Infectivity of Foreign Malarias to Anophelines of the Southern United States. (12 min) (Lantern) MARTIN D. YOUNG, TRAWICK H. STUBBS, JOHN M. ELLIS, ROBERT W. BURGESS, AND DON E. EYLES, United States Public Health Service.

26. Vectors of Scrub Typhus. (15 min) (Lantern) G. W. WHARTON, U. S. Naval Medical Research Unit No. 2 and Duke University.

27. Laboratory Studies on the Snail Host of *Schistosoma mansoni*. (15 min) (Lantern) ELOISE B. CRAM AND VIRGINIA S. FILES, National Institute of Health.

28. A Group Research Project on Biological Phases of *Schistosoma japonicum* and *S. mansoni* Infections. (15 min) (Lantern) WILLARD H. WRIGHT, National Institute of Health.

FRIDAY MORNING SESSION, MARCH 29, 11:00 AM; MUNICIPAL AUDITORIUM.

*Presidential Address*

29. The Making of a Parasitologist, ASA C. CHANDLER, Rice Institute.

FRIDAY, MARCH 29.

12:30 PM. PARASITOLOGISTS' LUNCHEON.

1:30 PM. ANNUAL BUSINESS MEETING.

FRIDAY AFTERNOON SESSION, MARCH 29, 3:00 PM. (TEA WILL BE SERVED).

*By Demonstration*

21. The Life History Cycle of *Diectophyma renale*, the Giant Kidney Worm of Man and Many Other Mammals. (Also read) ARTHUR E. WOODHEAD, University of Michigan.

30. Sarcocystis in the Monkey. A Report of Two Cases. EDWARD P. OFFUTT, JR. AND IRA R. TELFORD, University of Rochester and George Washington University.

31. Liver Coccidiosis Prevented by Sulfasuxidine. HARRY A. JANKIEWICZ, College of Osteopathic Physicians and Surgeons, Los Angeles.

32. A New Species of *Opecoeloides* (Trematoda: Opecoelidae) from the Threadfin Fish, *Polynemus octonemus*. JANE HOGAN VON WICKLEN, University of Nebraska. (Introduced by H. W. Manter.)

33. Tumor Formation as a Reaction to *Litomosoides carinii*, a Filariid of the Cotton Rat. J. ALLEN SCOTT AND JOY BARNES CROSS, University of Texas.

34. The Application of Cytological Techniques to Cestode and Other Helminth Material. ARTHUR W. JONES AND HELEN L. WARD, University of Tennessee.

51. Motion Picture of *Cercaria clausii*. Monticelli, a Marine Rattenkönig Larval Trematode from the West Coast of Florida. (Also read) RAYMOND M. CABLE AND R. A. McLEAN, Purdue University and Academy of Natural Science of Philadelphia.

52. Schistosomiasis. (Also read) PAUL P. WEINSTEIN, U. S. Public Health Service.

53. The Laboratory Diagnosis of Schistosomiasis. (Also read) M. M. BROOKE, U. S. Public Health Service.

SATURDAY MORNING SESSION, MARCH 30, 9:00 AM; MUNICIPAL AUDITORIUM.

Read

35. Preliminary Report on the Distribution of *Onchocerca cervipedis*. (15 min) (Lantern) CARLTON M. HERMAN AND ARTHUR I. BISCHOFF, California Division of Fish and Game.

36. The Rate of Growth and Maturity of *Litomosoides carinii*, a Filariid of the Cotton Rat. (15 min) (Lantern) J. ALLEN SCOTT, University of Texas.

37. Anatomical Studies on the Fourth Stage Larvae and Adults of *Litomosoides carinii*, a Filariid of the Cotton Rat. (15 min) (Lantern) JOY BARNES CROSS AND J. ALLEN SCOTT, University of Texas.

38. Treatment of Canine Filariasis with Trivalent Arsenicals (P-Arseno-Benzamides). (15 min) (Lantern) GILBERT F. OTTO AND THOMAS H. MAREN, Johns Hopkins University.

39. The Treatment of Human Filariasis (*Wuchereria bancrofti*) by Administration of Melarsen Oxide. (15 min) (Lantern) HARRY M. ROSE AND JAMES T. CULBERTSON, Columbia University.

40. Studies of Sheep Parasites. VI. Observations on Weather in Relation to Untreated Nematode Infections. (10 min) (Lantern) PHILIP A. HAWKINS, Michigan State College.

41. Immunity to *Necator americanus* Infection. (10 min) PAUL C. BEAVER, Tulane University.

42. Infection Experiments with a Hookworm of the Cotton Rat. (10 min) (Lantern) LYELL J. THOMAS, University of Illinois.

43. Hydrogen Ion Concentration as a Factor in Age Resistance to the Fowl Ascarid. (12 min) (Lantern) B. B. RIEDEL AND J. E. ACKERT, Kansas State College.

44. An Anomalous Experience with Phenothiazine. (15 min) (Opaque projection) PAUL D. HARWOOD, Dr. Hess and Clark, Inc., Ashland, Ohio.

SATURDAY AFTERNOON SESSION, MARCH 30, 2:00 PM; MUNICIPAL AUDITORIUM.

Read

45. Studies on the Cystochrome Oxidase of the Pig Ascaris. (10 min) (Lantern) C. A. HERRICK AND MARIE THEDE, University of Wisconsin.

46. Additional Studies on the Effects of Nodular Worm Infections on Calves During the Prepatent Period. (15 min) ROY L. MAYHEW, Louisiana State University.

47. The Relationship in Mice of Intestinal Emptying Time and Natural Resistance to *Hymenolepis*. (10 min) (Lantern) JOHN E. LARSH, JR., University of North Carolina.

48. Cercarial Production in Snails and Metacercarial Infections in Fish from Carrol Lake, Wisconsin. (15 min) L. O. NOLF, University of Wisconsin, Wisconsin Conservation Department, and University of Iowa.

49. Ecology of the Metacercariae of *Fasciola hepatica* in Southern Texas and Its Relationship to Liver Fluke Control in Cattle. (15 min) (Lantern) O. WILFORD OLSEN, U. S. Bureau of Animal Industry.

50. Determination of the Life History of *Cercaria szidati*, a Furcocercous Larval Trematode of the *Vivax* Type. (10 min) (Lantern) DORCAS J. ANDERSON, Purdue University.



51. Motion Picture of *Cercaria clausii* Monticelli, a Marine Rattenkönig Larval Trematode from the West Coast of Florida. (5 min) (Motion picture) RAYMOND M. CABLE AND R. A. McLEAN, Purdue University and Academy of Natural Science of Philadelphia.

52. Schistosomiasis. (15 min) (Motion picture) PAUL P. WEINSTEIN, U. S. Public Health Service.

53. The Laboratory Diagnosis of Schistosomiasis. (15 min) (Motion picture) M. M. BROOKE, U. S. Public Health Service.

*By Title*

54. Comparison of Methods for Diagnosing Schistosomiasis *japonica* by Recovery of Eggs from Feces. GEORGE W. HUNTER, III, JAMES W. INGALLS, AND MINNA G. COHEN, Army Medical School.

55. Periodicity of Microfilariae in the Philippines. J. L. AVERY, Naval Medical School.

56. The Thermal Death Point of *Cysticercus bovis*. REX W. ALLEN, U. S. Bureau of Animal Industry.

57. The Nature and Mechanism of Encapsulation in Trichiniasis. WAYNE W. WANTLAND, CORRINE L. BARDES, AND ROBERT S. LEVINE, Illinois Wesleyan University.

58. On the Distribution of Glycogen in the Trematode, *Ostiolum* sp. JAMES H. WILMOTH AND RHODA GOLDFISCHER, Brooklyn College.

59. Studies on the Respiration of the Frog Lung Fluke, *Ostiolum* sp. JAMES H. WILMOTH AND NORA LEVITAS, Brooklyn College.

60. A Note on the Genus *Axine* Abildgaard (Trematoda: Monogenea). EMMETT W. PRICE, U. S. Bureau of Animal Industry.

61. Additional observations on the Life History of *Gorgoderia amplicava* Looss, 1899. CHAUNCEY G. GOODCHILD, Southwest Missouri State College.

62. Studies on Parasites of Bassalian Fishes. ROSS F. NIGRELLI, New York Aquarium and New York Zoological Society.

63. Parasite Studies of Quail, *Colinus virginianus* and *Colinus virginianus texanus*, in Mississippi. J. W. WARD, Mississippi State College.

64. The Nematode Parasites of the Bufoninae (Amphibia-Salientia-Procoela-Bufonidae), I. A. C. WALTON, Knox College.

65. The Nematode Parasites of the Bufoninae, II. A. C. WALTON, Knox College.

66. The Nematode Parasites of the Bufoninae, III. A. C. WALTON, Knox College.

67. The Clinical Diagnosis of Schistosomiasis *mansoni* by Rectoscopic Biopsy. F. HERNANDEZ MORALES AND JOSÉ F. MALDONADO, University Hospital and School of Tropical Medicine, San Juan, Puerto Rico.

68. Schizomycetes parasitic in *Paramoecium bursaria*. RALPH WICHTERMAN, Temple University.

69. Chemotherapy of Filariasis (*Litomosoides carinii*) in the Cotton Rat by the Administration of Stibanose (= Solustibosan). JAMES T. CULBERTSON AND ELIZABETH PEARCE, Columbia University.

## AUTHOR INDEX

Showing program number, which is also abstract number of each paper.

<i>Author</i>	<i>Program and Abstract Number</i>	<i>Author</i>	<i>Program and Abstract Number</i>
Ackert, James E. ....	43	Levine, Robert S. ....	57
Allen, Rex W. ....	56	Levitas, Nora ....	59
Anderson, Dorcas J. ....	50	Lund, Everett E. ....	17
Avery, John L. ....	55	Maldonado, José ....	67
Bardes, Corinne L. ....	57	Maren, Thomas H. ....	38
Beaver, Paul C. ....	41	Mayfield, M. Frances ....	3
Bischoff, Arthur I. ....	35	Mayhew, Roy L. ....	46
Bozicevich, John ....	23	Maxwell, N. du Bose ....	19
Brackett, Sterling ....	15	McLean, R. A. ....	51
Bradford, Mary Jane ....	6	Morgan, Banner B. ....	5
v. Brand, Theodor ....	18	Nigrelli, Ross F. ....	62
Brooke, M. M. ....	53	Nolf, L. O. ....	48
Burgess, Robert W. ....	25	Offutt, Edward P., Jr. ....	30
Byrd, Elon E. ....	22	Olsen, O. Wilford ....	49
Cable, Raymond W. ....	51	Otto, Gilbert F. ....	38
Chandler, Asa C. ....	29	Pearce, Elizabeth ....	69
Coatney, G. Robert ....	10, 11, 20	Porter, Richard J. ....	12, 13
Cohen, Minna G. ....	54	Price, Emmett W. ....	60
Cooper, W. Clark ....	10, 20	Reardon, Lucy V. ....	3, 18
Coulston, Frederick ....	8	Rees, Charles W. ....	3, 18
Cram, Eloise B. ....	27	Riedel, B. B. ....	43
Cross, Joy Barnes ....	33, 37	Rose, Harry M. ....	39
Culbertson, James T. ....	39, 69	Scott, J. Allen ....	33, 36, 37
Dusseau, Elizabeth ....	12	Simpson, William F. ....	18
Ellis, John M. ....	25	Spindler, Lloyd A. ....	24
Engley, Frank B., Jr. ....	4	Stabler, Robert W. ....	4
Eyles, Don E. ....	25	Stubbs, Trawick H. ....	25
Fallis, A. Murray ....	9	Thede, Marie ....	45
Felsenfeld, Oscar ....	1	Telford, Ira R. ....	30
Files, Virginia ....	27	Thomas, L. J. ....	42
Goldfischer, Rhoda ....	58	Waletzky, Emanuel ....	15, 16
Goodchild, Chauncey ....	61	Walton, A. C. ....	64, 65, 66
Harwood, Paul D. ....	44	Wantland, Wayne W. ....	57
Hauschka, Theodore S. ....	19	Ward, Helen L. ....	34
Hawkins, Philip A. ....	40	Ward, James W. ....	63
Herman, Carlton M. ....	35	Warren, Virginia G. ....	23
Hernandez Morales, F. ....	67	Weinstein, Paul P. ....	52
Herrick, Chester A. ....	6, 45	Wharton, G. W. ....	26
Hershberger, Lloyd R. ....	11	Wichterman, Ralph ....	68
Huff, Clay G. ....	8	v. Wicklen, Jane Hogan ....	32
Hughes, Carrie Ola ....	16	Wilmoth, James H. ....	58, 59
Hunter, George W., III ....	23, 54	Wolfson, Fruma ....	14
Ingalls, James W. ....	54	Woodhead, Arthur E. ....	21
Jankiewicz, Harry A. ....	7, 31	Wright, Willard H. ....	28
Johnson, Eleanor M. ....	3, 19	Young, Martin D. ....	25
Jones, Arthur W. ....	34	Young, Viola Mae ....	2
Laird, Raymond L. ....	12, 13	Zimmerman, Harry E., Jr. ....	24
Larsh, John E., Jr. ....	47		

1. *Comparison of Methods Used for the Detection of Endamoeba histolytica.* OSCAR FELSENFELD, Mount Sinai Medical Research Foundation, Chicago.

317 diarrheic stools were examined with the aid of direct smears, suspensions treated with D'Antoni's iodine, Quenzel's stain, zinc flotation, cultivation in Locke-egg-serum and liver-serum, and permanent hematoxylin-stained slides.

103 stools were found to contain *Endamoeba histolytica*. The probability of the detection of this protozoön was 0.41 using direct smears and D'Antoni's iodine; 0.43 with Quenzel's stain; 0.66 in culture; 0.88 with hematoxylin-stained slides and 0.93 using zinc flotation. In stools containing many cellular elements, e.g., proctoscopic specimens, the probability of a positive result using hematoxylin-stained slides increased to 0.91 and that of zinc flotation decreased to 0.81. Because of the easy preparation of hematoxylin-stained slides and their permanent value, this method is recommended for general use, supplemented by zinc flotation in cases showing no amoebas or cysts upon examination of permanent slides.

2. *The Staining of Protozoa in Formalized Stool Specimens.* VIOLA MAE YOUNG, Mount Sinai Medical Research Foundation, Chicago.

The preservation of stool specimens with 10 per cent formalin holds forth much promise as an aid when parasitologic examination cannot be immediately performed. Such specimens can be used for direct examination and salt flotation. Degenerative forms of protozoa, however, require a hematoxylin stain for reliable diagnosis. Heretofore means of staining formalized intestinal protozoa have not proved satisfactory. In order to surmount this technical gap, the hematoxylin stains of Heidenhain, Delafield, Harris, Weigert, and Mallory followed by differentiating methods of Johnson, van Gieson, and Ratcliffe were investigated. It was found that the most reliable results were achieved by staining the specimens in bulk. Washing out of the fixative, staining, differentiation and dehydration are carried out by successive centrifuging, decanting and resuspending in the solutions to be employed. Mallory's phosphomolybdic hematoxylin for 18 to 24 hours followed by differentiation with distilled water gave the most satisfactory results. The stained specimens are finally treated with two successive changes of xylol and suspended in clarite. The latter suspension is mounted, using pressure on the coverslip during the drying process to avoid too much variation in depth.

3. *The Influence of Whole Egg Inoculum on the Growth of Endamoeba histolytica-organism t in Enriched Egg-White Medium and in Whole-Egg-Dialysate Medium.* CHARLES W. REES, LUCY V. REARDON, ELEANOR M. JOHNSON, AND M. FRANCES MAYFIELD, National Institute of Health.

Egg-white medium enriched with 9 vitamins of the B group, cholesterol, and rice powder produced good growth of *Endamoeba histolytica*-organism *t* when inoculated from whole-egg medium but was deficient for amoeba-growth on serial transfer. The growth of organism *t* continued at a high level in serial transfer. Further enrichment of egg-white medium with casein hydrolysate and purine bases was without effect on the growth of the organisms. When inoculated from whole-egg medium good growth of *E. histolytica* occurred in loops of cellophane tubing containing rice powder and suspended in the overlay of whole-egg medium. As in the case of enriched egg-white medium the amoebae died out in serial transfer. The growth of organism *t* was not measured under these conditions. These data indicate that certain growth factors in egg white, egg yolk, and rice powder are essential for the growth of *E. histolytica* in the presence of organism *t*. The enrichment formulae added to egg-white medium failed to supply all of the missing yolk factors. The latter occur in whole-egg inoculum of *E. histolytica*-organism *t* but are absent in whole-egg dialysates of cultures of these two organisms.

4. *Pathogenicity of Pure Culture Trichomonas gallinae Orally Administered to Clean Pigeons.* ROBERT M. STABLER AND FRANK B. ENGLEY, JR., University of Pennsylvania.

The authors performed 42 implantations in studying the host-parasite relations of *T. gallinae* in birds from the senior author's loft of pigeons free of this pathogen. The material was obtained from a falcon (*Falco peregrinus anatum*) which died of infection with *T. gallinae*. Five types of inoculum were used: 1. Bacteria from the falcon's lesions (*Escherichia coli communis* and *Micrococcus candidans*); 2. Bacteria in the protozoan cultures from the falcon's lesions (the above plus *Bacillus subtilis*); 3. The protozoan culture (*T. gallinae* plus the bacteria in 2); 4. Cultures 1 and 3 in combination; 5. The falcon's *T. gallinae* in pure culture. In all experiments a 1-cc suspension of fresh inoculum was merely dropped by pipette into the back of the pigeon's open mouth. No injections were given. Of the 12 birds receiving bacteria alone none was affected; of the 17 receiving pure culture *T. gallinae* 15 died, 2 were used for other purposes;

in the 13 receiving *T. gallinae* and bacteria the lesions were no different in extent or kind from those receiving *T. gallinae* alone. Of the total of 30 birds given *T. gallinae* 4 were sacrificed for study and 26 ran a natural course. Of these latter, 21 died and 5 recovered. Lesions appeared in the mouth in 5.1 days (average). They were common in the mouth, oesophagus, lower crop, and liver. Various involved were intestinal surface, pancreas, heart, air sacs, the bone of the floor of the skull, and the tissues of the eye. Death occurred in 9.8 days (average).

5. *Further Studies on the Inoculations of Trichomonas foetus (Protozoa) in Heifers.*

BANNER BILL MORGAN, University of Wisconsin.

In a previous work, 9 heifers were injected intramuscularly or intravenously with 5 ml of living, washed, bacteria-free *Trichomonas foetus* approximately twice per week for a period of 3 months. These animals were apparently protected against bovine trichomoniasis as they could not be infected by way of the genital tract. Nine controls developed various manifestations of the disease. Further studies were continued using 20 virgin heifers (10 test animals and 10 controls). Two injections of 5 ml each of living trichomonads (50 million organisms per ml) were given during a period of 3 weeks. Immobilization of the trichomonads in dilutions ranging from 1:4 to 1:8 was observed in the sera of these heifers. At the first heat period after the last injection each cow was artificially inseminated with fresh normal semen. Twenty-four hours later all cows were inoculated in each uterine horn with 10 ml of motile *T. foetus* with 10 million organisms per ml. Ten control heifers were not vaccinated. Of the 10 vaccinated cows 8 showed heavy trichomonad infections and only 2 became pregnant. In the control group 4 animals were positive for *T. foetus* and 7 became pregnant. The results demonstrated that 2 intramuscular injections of *T. foetus* within a period of 3 weeks were not sufficient for protection against bovine trichomoniasis.

6. *Toxicity of Cecal Cores from Chickens Infected with the Protozoan, Eimeria tenella.*

MARY JANE BRADFORD AND C. A. HERRICK, University of Wisconsin.

It was found that the blood and cellular debris from the cecal hemorrhage caused by *Eimeria tenella* produced intravascular coagulation and death in chickens when injected intravenously. This thromboplastic effect was apparently limited by the appearance of blood in the cecal material, since saline extracts of the contents present from the first to the fourth day after infection caused no intravascular coagulation and were lethal in only five out of twenty chickens. The injection of these extracts and ones from normal cecal contents caused regurgitation and weakening, but ordinarily the chickens recovered. On the other hand, similar extracts of the bloody material which appeared after the cecal cells were ruptured on the 5th day were invariably fatal within one minute after intravenous injection. Extracts of the cores formed on the 6th and those that were retained until the 13th day were similarly fatal. If chickens were autopsied immediately, large clots were found in the veins and heart.

This thromboplastic effect could be neutralized by an anti-serum developed in rabbit against chicken cecal mucosa.

It is suggested that, since thromboplastic material may be absorbed from the site of tissue injury, it may account, at least in part, for the death resulting from cecal coccidiosis.

7. *Dosage of Eimeria stiedae Related to Severity of Liver Coccidiosis.* H. A. JANKIEWICZ, College of Osteopathic Physicians and Surgeons, Los Angeles.

Male domestic rabbits 9 weeks old, 3 to a group, were given each 0, 100, 1,000, 10,000 or 100,000 sporulated oöcysts. Twenty-two days later the rabbits were autopsied. When the total weight of the livers of each group was calculated as the percentage of total body weight for that group, the respective percentages for the dosages were 3.6, 4.3, 7.5, 11.7 and 16.0. In one rabbit of the 100,000 group the liver constituted 20.5% of its body weight. Correlated with the relative increase of liver size and weight, there was a corresponding increase of the number of gross lesions of the bile ducts and a decrease of normal liver tissue visible on the surface. The enormous increase in the amount of biliary epithelium and the proliferation of new bile ducts primarily caused the enlargement of the liver.

Beginning with the 100 dosage, the percentage of normal sporulation of oöcysts secured from the bile of the gall bladders was respectively: 76, 65, 43 and 14. Therefore lightly infected livers are a better source of normal oöcysts than are heavily infected ones. The oöcysts first appeared in the feces 16 to 17 days after the date of inoculation. By the twenty-second day the schizogony phase has nearly been completed; most of the coccidia are undergoing gametogenesis or have reached the oöcyst stage.

8. *Cryptozoites and Metacryptozoites of Plasmodium relictum in Canaries and Pigeons.* FREDERICK COULSTON AND CLAY G. HUFF, University of Chicago.

Using the methods described recently (1944) by us for the demonstration of the development of sporozoites of *P. gallinaceum* into pre-erythrocytic stages, it was possible to prove the existence



of cryptozoites and metacryptozoites in *P. relictum* of canaries and pigeons. Four strains of *P. relictum* were used; namely, a newly isolated strain (1Q), Coatney's pigeon strain (1P) not transmissible by mosquitoes, Redmond's strain of 1P adapted to canaries (1P1) and transmissible by *Culex*, and Redmond's strain of 1P1 after mosquito passage (1P1-1) which does not produce erythrocytic infections in pigeons either by blood or sporozoite inoculation.

Pre-erythrocytic stages were demonstrated in the 1Q, 1P1, and 1P1-1 strains. These cryptozoites and metacryptozoites, although similar to *P. gallinaceum* in types of cells infected and rate of development from the sporozoite to cryptozoic segmenter, differ somewhat in morphological appearance. The merozoites are rounder instead of being pointed as in *P. gallinaceum* and there are fewer per segmenter. Often individual parasites stain pink rather than blue, a phenomenon never observed in *P. gallinaceum*.

Of special interest is the fact that cryptozoites, metacryptozoites and erythrocytic stages were found in canaries infected with sporozoites of strains 1P1 and 1P1-1 of *P. relictum* but only pre-erythrocytic stages could be demonstrated in the pigeon. However, upon subinoculation of blood from infected pigeons to canaries, many of the canaries developed typical parasitemia thus proving the existence of subpatent infections in the pigeons. (Work done under contract with the Office of Scientific Research and Development.)

9. *Plasmodium circumflexum* in the Ruffed Grouse in Ontario. A. MURRAY FALLIS, Ontario Research Foundation, Toronto.

A species of plasmodium which was discovered in the ruffed grouse in Ontario appears to be a strain of *P. circumflexum*, although, when studied in the grouse only, it had characters midway between those of *P. lophurae* and *P. circumflexum*. It has been transferred to grouse, canaries, turkeys, and ducks. There was no indication that it was pathogenic to any of these birds. Development occurred more readily in grouse than any of the above-mentioned birds. Parasites were present in the peripheral blood of a grouse for at least 8 months following infection. The infection was observed from 2 to 15 days in canaries with the exception of one in which it remained for 40 days. It appeared in the peripheral circulation in one of the ducks for 6 days and in the turkeys for 2 days only. Infections in the grouse reached higher parasite levels than in the other birds. No well-defined periodicity or synchronicity was observed.

The variations in the morphology and behavior of the parasite which occur following repeated transfers from canary to canary have been followed and the parasite has been compared with another strain of *P. circumflexum* and with *P. lophurae*.

10. *Studies on Plasmodium gallinaceum* Brumpt. IV. *Parasitemia and Survival in Blood-Induced Infections in Young Chicks*. W. CLARK COOPER AND G. ROBERT COATNEY, National Institute of Health.

During 4 years of screening potential antimalarial drugs, over 22,000 week-old White Rock chicks maintained on a formulated stock diet were given standard intravenous inoculations of  $16 \times 10^6$  red blood cells containing *Plasmodium gallinaceum*. Of these, 2,000 were untreated controls. The basic data used for drug appraisal (4th-day-parasitemia and survival) have been analyzed. In the controls, 4th-day parasitemia averaged 5,440 parasitized red blood cells per 10,000. Over 99% of these control birds died of the acute infection, the periods of survival showing a characteristic biphasic pattern, with frequency peaks at 6 and 17 days. Even after active drugs, mortality was only slightly decreased, though a greater proportion of deaths occurred in the late phase.

Data are also presented from a smaller series to show the variations that occur even with standardized procedure and the use of a single suspension of parasitized cells; results in another series show the effect of varying the size of the inoculum, by serial dilution, from  $10^8$  to  $10^{-1}$  parasitized red cells. (Work done under contract with the Office of Scientific Research and Development.)

11. *Studies on Plasmodium gallinaceum* Brumpt. VI. *A Study of the Pathology in Young Chicks*. LLOYD R. HERSHBERGER AND G. ROBERT COATNEY, National Institute of Health.

A lot of 130 week-old White Rock chicks were each given  $16 \times 10^6$  red cells parasitized by *P. gallinaceum* and then killed in numerical order on the 2, 4, 5, 6, 7, 8, 10, 12 and 14th day after inoculation for a study of the progressive histopathology correlated with the general course of the disease. Data are also presented on the physiochemical characteristics of the pigment.

Parallel data are presented from a similar lot of chicks each given "one mosquito equivalent" of sporozoites subcutaneously and then killed in numerical order. (Work done under contract with the Office of Scientific Research and Development.)

12. *In vitro Studies on Malarial Sporozoites*. RICHARD J. PORTER, RAYMOND L. LAIRD, AND ELIZABETH DUSSEAU, University of Michigan.

The increasing laboratory use of mosquito-induced malarias demands reliable procedures for

infecting experimental animals with sporozoites. To this end, various methods of introducing *Plasmodium gallinaceum* sporozoites into chickens have been evaluated.

Data are reported concerning methods of immobilizing mosquitoes for dissection, media and temperatures for suspension of infected salivary glands, techniques for estimating sporozoite dosage, and routes of inoculation of sporozoite suspensions into chickens. On the basis of these data, the following procedure is recommended: Infected mosquitoes are killed with cigarette smoke. The salivary glands are removed in normal saline and ground in a roller mill containing heparinized normal chicken blood. The concentration of sporozoites is estimated from stained dry smears of this suspension. The suspension is diluted with normal saline solution and inoculated intravenously. (The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Michigan.)

13. *Observations on the Courses of Infection in Sporozoite-Induced Plasmodium cynomolgi Malaria.* RAYMOND L. LAIRD AND RICHARD J. PORTER, University of Michigan.

In order to test drugs for possible curative action against malaria, it seemed desirable to accumulate a large series of sporozoite-infected test animals. *Plasmodium cynomolgi* was selected as an experimental organism because in some respects it apparently is similar to *Plasmodium vivax*, and it can be transmitted through laboratory strains of *Anopheles quadrimaculatus*.

In the observations reported in this paper, *Anopheles quadrimaculatus* were infected by feeding on infected monkeys. After a suitable incubation period, the salivary glands were dissected from the mosquitoes and suspended in monkey serum or heparinized monkey blood. This suspension was then ground in a roller mill, diluted with serum and saline, and inoculated into the monkeys intravenously. Observations were then made concerning the prepatent period, patent period, and relapse rate in treated and untreated infections. (The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Michigan.)

14. *Plasmodium elongatum in Pekin Ducks.* FRUMA WOLFSON, Johns Hopkins University. *P. elongatum* was reintroduced into the duck in order to make it available for chemotherapy research. The present strain is designated as "5 E" and is different from the one previously studied in the duck.

The parasite was inoculated into the duck from a canary and was subsequently passed through 40 consecutive semi-weekly intravenous subinoculations. A total of approximately 130 birds was used. Eighty of these were studied throughout the patent period of infection. Judging from observations of the peripheral blood, rapid passage through the duck did not raise the degree of parasitemia. The percentage of parasitized erythrocytes was usually under 10 at the peak of infection, which occurred on the third day after inoculation with 3 billion parasites per kilogram of duck weight. In the surviving birds this peak was followed by the second one—sometimes as high as the first. Of 80 birds inoculated with doses up to 3 billion parasites 74% died during the first two weeks after inoculation. Deaths were apparently due to rapidly developing anemia. The RBC count dropped from over 2.5 million per cu mm before inoculation to less than 0.5 million six days after inoculation. Such severe anemia was probably caused by the large numbers of parasites found in locations other than peripheral blood, particularly bone marrow. The erythrocytes or their precursors were probably destroyed before they had a chance to enter the peripheral circulation. In spite of an abundance of gametocytes, attempts to infect *Culex pipiens* thus far have failed. The parasite was successfully preserved by low temperature freezing. (This work was done under contract with the Office of Scientific Research and Development.)

15. *The Relations between Pantothenic Acid and Plasmodium gallinaceum Infections of the Chicken.* STERLING BRACKETT AND EMANUEL WALETZKY, American Cyanamid Company, Stamford, Connecticut.

The course of blood-induced *Plasmodium gallinaceum* infections in the chicken is greatly altered when the host is maintained on a diet deficient in pantothenic acid. When relatively small inocula of parasitized erythrocytes are used, peak parasitemia is much lower in pantothenic acid deficient birds than in birds on a supplemented diet, and the average number of merozoites per schizont is reduced in the deficient birds. This suggests that the malaria parasite requires pantothenic acid. However, the course of sporozoite-induced infections is not materially altered by a pantothenic acid deficiency. Similarly some analogues of pantothenic acid, when administered in the diet, are highly effective in suppressing blood-induced infections, but do not suppress sporozoite-induced infections. This refractory behavior of sporozoite-induced infections may be associated with the predominantly non-erythrocytic development of the parasites in the early part of such infections, and the comparatively high concentrations of pantothenic acid in tissues

other than blood, rather than with a difference in the pantothenic acid requirements of the erythrocytic and the various exoerythrocytic stages of the malaria parasite.

16. *The Relative Activity of Sulfanilamides and Other Compounds in Eimeria tenella Infections of the Chicken.* EMANUEL WALETZKY AND CARRIE OLA HUGHES, American Cyanamid Company, Stamford, Connecticut.

The activity of sulfaguanidine in *Eimeria tenella* infections of the chicken was strikingly surpassed by only a few of the forty-five other sulfanilamides tested, namely: the halogenated sulfapyrimidines, sulfamethazine, and sulfapyrazine which were respectively about ten, five and four times as active as sulfaguanidine on a dosage basis. The activity of sulfanilamides in coccidiosis resembles that in other infections since it is antagonized by para amino benzoic acid, and is dependent upon the presence of a free amino group in the para position of the benzene ring. Since there is some correlation between the activity of sulfanilamides in the coccidiosis and the malaria of the chicken, some thirty-nine other sulfanilamides which show only slight antimalarial activity in the chicken are probably not promising coccidiostatic agents. No correlation between antimalarial and anticoccidial activity exists in other types of compounds since such potent antimalarials as quinine, atabrin, and plasmochin are inactive against coccidiosis. Poorly absorbed sulfanilamides showed little or no anticoccidial activity, and the effect of active sulfanilamides paralleled their blood concentrations. This may be related to the slow rate of action of the sulfanilamides and the brief duration of the extracellular stages of the coccidia. Some of the sulfanilamides which are superior to sulfaguanidine in avian coccidiosis may be even more strikingly superior in mammalian coccidiosis since sulfaguanidine maintains much higher blood concentrations in birds than in mammals.

17. *Aspects in the Control of Intestinal Coccidiosis in Commercial Rabbitries by the Use of Phthalylsulfathiazole.* EVERETT E. LUND, United States Rabbit Experiment Station, Fontana, California.

Phthalylsulfathiazole has been used in doses averaging 0.20 to 0.25 grains per pound of body weight per day, in an effort to develop an economically practical means of controlling intestinal coccidiosis in commercial rabbitries. In addition to varying the time and manner of administering the drug, it has been used on animals of various ages, animals with varying degrees of active acquired immunity, and on animals with experimentally produced infections with both fresh and "time-attenuated" strains.

Determination of the effectiveness of the drug under the various circumstances was based on suppression of oöcysts capable of sporulation, gross pathology, weight changes, ability to produce and rear litters, and on dressing percentages.

Most tests dealt with infections with *Eimeria magna*. Random observations of the effects on other forms were recorded, and comparison to the use of succinylsulfathiazole in some instances was indicated.

18. *Chemical Studies on Egg-White Medium for the Cultivation of Endamoeba histolytica.* THEODOR VON BRAND, CHARLES W. REES, LUCY V. REARDON, AND WILLIAM F. SIMPSON, Catholic University of America and National Institute of Health.

A diphasic medium dispensed in Florence flasks of 250-ml capacity as used for the cultivation of *Endamoeba histolytica*, consisting of a base of 50 ml of inspissated egg white with 3 gm of powdered egg shell, and an overlay of 200 ml of Locke's solution, was stored for periods up to 20 days and analyzed for reducing substances, fats, and nitrogenous compounds. The rates of diffusion were measured in the overlay and the results compared with analyses of egg-white emulsion. Diffusion of reducing substances was rapid, nearing completion after 2 days and 8 days at 37° C and 10° C, respectively. Diffusion of nitrogenous compounds was considerably slower and it had not reached a standstill at 20 days, although it was most rapid during the first 8 days. Only a trace of fats was demonstrable in the overlay and there was no evidence of diffusion. The reducing substances were composed primarily of glucose. The nitrogenous material was calculated as ovomucoid which is the only egg-white protein not coagulable by heat. Some of the reducing substances appeared to be indiffusible. Curves were plotted for standardization of medium for the cultivation of *E. histolytica*.

To determine the extent of spontaneous oxidations of the medium, air was confined over the base, with and without overlay, and analyzed for oxygen content after designated periods of storage. A decrease of oxygen occurred in the amount of 1 ml per 125 ml of air but no corresponding decrease in air over Locke's solution or in air-filled flasks was observed. The data are in agreement with a theory that components of egg white contributing to the growth of *E. histolytica* may be rendered inactive by oxidation. There was some increase of carbon dioxide content of air over the medium but comparative figures showed no consistent trends.



19. *Hexenolactone, an Effective Inhibitor of Trypanosoma cruzi Growth in vitro, but not in vivo.* THEODORE S. HAUSCHKA, N. DU BOSE MAXWELL, AND ELEANOR M. JOHNSON, Lankenau Hospital Research Institute and National Institute of Health.

Hexenolactone (parasorbic acid) occurs as a natural growth-inhibitor in various plants and its synthetic equivalent has been shown to interfere with cellular multiplication in several bacteria, yeasts and animal tissues. Fourteen experiments involving five concentrations of the lactone (M/50,000, M/10,000, M/2000, M/1000 and M/600, the last being equivalent to a near-toxic concentration in mammals) and two culture media at like pH and temperature showed from partial inhibition to inactivation and death of *Trypanosoma cruzi* (Culbertson strain). Haemocytometer counts of the trypanosomes, occasionally checked by photo-electric turbidity comparisons, were made at regular intervals. A representative series of results with M/2000 hexenolactone follows: After 6 days of growth the tests showed under 1000 trypanosomes per cubic millimeter, the control counts were 34,000 35,000, 49,000. There was no test increase after 10 days, while the control counts had risen to 92,000, 96,000 and 99,000. After 20 days the picture was similar, but there were still some viable trypanosomes in the tests, as demonstrated by transfers to normal medium. The two higher concentrations tested killed the organisms after 4-10 days exposure.

Experiments on 76 rats and mice, infected with *T. cruzi* or *T. equiperdum* and injected either intraperitoneally or intravenously with near-toxic amounts of hexenolactone, gave no trypanocidal results of clinical significance. This discrepancy between *in vitro* and *in vivo* effects is in keeping with the sulfhydryl-conditioned inhibitory mechanism of hexenolactone (Hauschka, Toennies and Swain, 1945, Science 101: 283-285, reduced sulfhydryl compounds being constantly replenished in the living host, but not in culture media.

20. *Studies on Plasmodium gallinaceum Brumpt. V. Quinine Standardization in Screening Tests Against Blood-Induced Infections in Young Chicks.* G. ROBERT COATNEY AND W. CLARK COOPER, National Institute of Health.

Quinine was used as a standard in quantitative appraisal of suppressive activity against blood-induced *P. gallinaceum* infections in week-old White Rock chicks. Each bird received medication by mouth twice daily for 4 days with intravenous inoculation of  $16 \times 10^6$  parasitized red cells approximately 6 hours after the first dose of drug. The evaluation of activity was based on the average parasite count in the 5 birds constituting a test group, on the morning after last medication. The smallest dosage which reduced the average 4th day parasitemia to 25% or less of that in the controls was considered the minimum effective dosage.

Data are presented to show that the minimum effective dosage of quinine base is approximately 0.016 mg/g. A series of 220 birds inoculated from a single suspension is presented, in which 55 birds were given 0.016 mg/g of quinine base, 55 were given 0.008 mg/g, and 110 served as untreated controls; the variations in parasitemia at threshold dosages are shown.

Two quinine-treated groups, given 0.016 and 0.008 mg/g respectively, were included as controls in routine drug series. Data on approximately 1000 such quinine-treated birds in 100 consecutive drug series, are analyzed. In nearly 90% of the series the higher dosage was effective, the lower was not. Data on the minimum effective dosages of quinacrine (atabrine) and plasmochin under similar conditions are included for comparison. (Work done under contract with the Office of Scientific Research and Development.)

21. *The Life-History Cycle of Dioctophyma renale, the Giant Kidney Worm of Man and Many Other Mammals.* ARTHUR E. WOODHEAD, University of Michigan.

Eggs when incubated at room temperature for six months contain infective larvae. The larva hatches after a few minutes in the fore-gut of branchiobdellids which are parasitic on crayfish. Penetration into the body-cavity follows in about ten minutes and the larva soon comes to rest in various organs or tissues. About ten days are required to transform into a shorter, gordius-like larva. A cyst is formed which is easily confused with the cyst of the Gordiacea.

The northern black bullhead, *Ameiurus melas melas*, eats branchiobdellids from the bottom or consumes the branchiobdellid-laden exuviae of crayfish. The excysted larva migrates to the mesenteries and again encysts. The larva then undergoes extensive linear growth similar to the growth of *Paragordius varius* in crickets. The head of the second stage larva shrivels and a new third stage (or final) head and characters of the final adult become apparent.

Two ferrets were fed bullheads collected in July from an infected area. This feeding resulted in the death of one ferret four weeks later, from which a 30-mm *D. renale* female was recovered, and the illness of the second which is alive as of November 15. Sectioned mesenteries from this and later collections of bullheads showed many *D. renale* cysts in all stages of development. A 10-mm male *D. renale* was recovered from a cyst in the mesentery over the stomach of a bullhead.

Bullheads become infected coincident with the molting of crayfish.

Two years are required to complete the life-cycle from egg to adult.



22. *Epidemiological Investigations on Filariasis on Certain Islands of the South Pacific Area.* ELON E. BYRD, University of Georgia.

From epidemiological studies on filariasis in the South Pacific Area it is shown that rarely are the microfilariae (*Wuchereria bancrofti*) found in the circulating blood of children under three years of age. Between the 3rd and 15th years of life the incidence of infection ranges around 5 per cent for the Polynesian and 10 per cent for the Melanesian children. The incidence of blood microfilariae more than doubles this level in the age group 16 to 20 years, and this per cent level steadily increases for both groups until more than 40 per cent of those individuals 50 years of age and over can be demonstrated (single smear method) to harbor the filaria larvae. There is a corresponding higher incidence of microfilaria-positive blood in all age groups in the Melanesian race than can be shown for the Polynesian. It is impossible to say whether this higher incidence level is due to race, species of parasite, physiology, or transmitting host. However, it is known that the aperiodic filaria occurs throughout Polynesia and that a periodic form parasitizes the Melanesian population. The aperiodic filaria is transmitted by *Aedes pseudoscutellaris*, a day feeding mosquito; while the periodic form is transmitted by a night feeding species, *Anopheles farauti*. Through field studies it was possible to demonstrate that up to 35 per cent of *A. pseudoscutellaris* from isolated village areas harbored developing filaria larvae, and in one village more than 80 per cent of *Anopheles farauti* carried these forms. Some evidence was obtained in Polynesia which indicated the rate of transmission was seasonal. Cross-infection experiments indicated that the transmission of filariasis is strictly a concern of the local species of mosquitoes, and that, although several species may be capable of completing the developmental cycle of the parasite, rarely can more than one or two species be demonstrated to be principal vectors within a given endemic area.

23. *Studies in Filariasis. II. A Skin Test for Filariasis bancrofti Utilizing Antigen Prepared from Microfilariae of Wuchereria bancrofti.* GEORGE W. HUNTER, III, JOHN BOZICEVICH, AND VIRGINIA G. WARREN, Army Medical School and National Institute of Health.

Sixty-five patients suspected of having filariasis were inoculated intradermally with antigens prepared from microfilariae of *Wuchereria bancrofti* and the adult dog heart worm, *Dirofilaria immitis*. In order to establish the optimum dilution for the *Wuchereria bancrofti* antigen, patients were inoculated with 1:1000, 1:4000, 1:8000, 1:12,000, and 1:16,000 and 1:20,000 dilutions of the material. These results were compared with those elicited by the *Dirofilaria immitis* antigen, the optimum dilution of which previously had been found to be 1:8000. In this series the *bancrofti* preparation appeared to be at least as sensitive as the *immitis* antigen, and probably the more specific.

In a control group of 33 non-allergic individuals which had never been exposed to filariasis, none reacted to the new antigen made from mf of *Wuchereria bancrofti*, and only four false positive reactions were encountered. No false positive responses occurred in 21 enlisted men harboring one or more of the following parasites: *Strongyloides stercoralis*, *Ascaris lumbricoides*, hookworm, *Hymenolepis nana* and *S. japonicum*. This specificity exhibited by the *bancrofti* antigen would certainly serve to enhance its value as a diagnostic aid in the diagnosis of filariasis.

24. *The Biological Status of Sarcocystis.* L. A. SPINDLER AND HARRY E. ZIMMERMAN, JR., U. S. Bureau of Animal Industry.

The biological status of parasites comprising the genus *Sarcocystis* has been somewhat in doubt. An investigation on the species infecting swine showed that this parasite is a fungus and not a protozoan as commonly supposed. The main points of the investigation are as follows:

Sarcocysts or Miescher's sacs, removed aseptically from the muscles of swine, were ruptured in sterile dextrose culture solution to release the spores; the preparations were cultured at 37° C for 24 hours and subsequently at room temperature. After a few days to two weeks of culturing, the spores budded off minute coccoid bodies which developed, by incomplete budding, into a septate mycelium with vertical hyphae bearing spores. The fungus has been identified as *Aspergillus* sp.

Twenty-five of 50 young pigs injected with, or fed, conidia harvested from the cultures harbored at necropsy, 4 to 6 months after infection, typical sarcocysts in the muscles; controls escaped infection. Cultures made from the mature sarcocysts yielded a fungus like that injected.

Pigs, rats, and mice, fed sarcocysts, discharged with their urine and feces yeast-like bodies which, in culture, produced a fungus like that originally cultured from sarcocysts. Sections of kidneys of infected mice showed yeast-like bodies in the tubules. In rats and mice clumps of a fungus were found attached to the walls of the ileum and cecum; yeast-like bodies similar to those found in feces and urine budded from the internal walls of this fungal growth.

25. *Studies on Imported Malaria: 4. The Infectivity of Foreign Malariae to Anophelines of the Southern United States.* MARTIN D. YOUNG, TRAWICK H. STUBBS, JOHN M. ELLIS, ROBERT W. BURGESS, AND DON E. EYLES, U. S. Public Health Service.

The infectivity of foreign malariae relapsing in returned soldiers to anophelines of the

Southern United States was investigated. A total of 173 lots of *Anopheles quadrimaculatus* was fed upon *Plasmodium vivax* relapsing in soldiers. The origins of the infections were: South Pacific—117; Mediterranean—40; Carribean—6; Liberia—1; Burma—1.

Malarias from each of the above areas infected *A. quadrimaculatus*. The rate of infection in the total 6,247 mosquitoes dissected was 30.8 per cent.

*A. quadrimaculatus* successfully transmitted these infections to man. These foreign malarias induced in neurosyphilitic patients were also infective to *A. quadrimaculatus*, and continued to be through several serial transfers both by mosquitoes and blood inoculations.

Gametocytes were produced and mosquitoes were infected as long as relapses occurred. The case with the most relapses (24) and the case with the longest duration of the disease (31 months) both infected mosquitoes.

The average length of the sporogonous cycle of *P. vivax* in *A. quadrimaculatus* was 10.7 days when incubated at 74–80° F.

Mosquitoes kept at outdoor temperatures in Texas during April and May developed infections at about the same rate as those kept in the insectary.

*A. punctipennis*, *A. p. pseudopunctipennis* and *A. albimanus* also became infected by the foreign malarias.

The foreign malarias studied readily infected *A. quadrimaculatus* and were transmitted by this vector.

26. *Vectors of Scrub Typhus*. G. W. WHARTON, U. S. Naval Medical Research Unit No. 2 and Duke University.

Trombiculid mites have long been recognized as vectors of scrub typhus or tsutsugamushi fever. There are well over a hundred species of the family Trombiculida reported from the areas in Australia, Asia, and the islands of the Pacific where the disease occurs in man. The mites that have been demonstrated to carry the disease all belong to the genus *Trombicula sensu strictu* and all are characterized in the larval (chigger) stage by having feathered rather than nude galeal setae; a nude seta on papal segment two; a nude seta on papal segment three; and a feathered dorsal, nude lateral, and nude ventral seta on papal segment four. At least one species of mite with the above characteristics has been found associated with scrub typhus in Japan, Formosa, Pescadores Islands, Malaya, Sumatra, India, New Guinea, Australia, Philippine Islands, and the Solomon Islands. No larval trombiculids with the above characteristics were found in areas in the Solomon Islands, Ryukyu Islands, Guam, Iwo Jima, Ulithi, and Peleliu where scrub typhus is not known to occur. It therefore seems probable that mites of the genus *Trombicula* that possess the morphological features described above play the part in transmission of scrub typhus that belongs to the anopheline mosquitoes in the transmission of malaria. The following names have been used for the eastern species of mites that have the above characteristics: *Trombicula akamushi*, *T. pallida*, *T. palpalis*, *T. scutellaris*, *T. intermedia*, *T. keunhenschrijveri*, *T. fletcheri*, *T. obscura*, *T. deliensis*, and *T. walchi*.

27. *Laboratory Studies on the Snail of Schistosoma mansoni*. ELOISE B. CRAM AND VIRGINIA S. FILES, National Institute of Health.

Using the Puerto Rican strain of *Schistosoma mansoni* and its known intermediate host, *Australorbis glabratus*, as a control, additional attempts have been made to infect snails native to the United States including members of the genera *Gyraulus*, *Helisoma*, *Planorbarius*, *Planorbula* and *Tropicorbis*. In a species of *Tropicorbis* originating from Louisiana sporocysts and cercariae developed. The cercariae proved infective for mice, causing lesions attendant to the development of the adult worms and the dispersal of ova comparable to those produced in controls with cercariae from *A. glabratus*. Cercarial production was continued in *Tropicorbis* for as long as 107 days, the daily output increasing as time went on, resulting in the death of the snail. A relatively small percentage of this species of snail proved susceptible to infection. A series of experiments was conducted to test the effect of various techniques of exposure, taking into consideration such factors as the species of mammalian host from which ova were derived, the age and size of the snail, whether laboratory reared or "wild," individual or mass exposures, and the effect of previous exposures.

28. *A Group Research Project on Biological Phases of Schistosoma japonicum and S. mansoni infections*. WILLARD H. WRIGHT, National Institute of Health.

Field observations made by the writer in the Philippine Islands were coordinated with laboratory studies made by other members of the staff at the Zoölogy Laboratory, National Institute of Health. Snails and mammals infected with *S. japonicum* were supplied by the Office of the Surgeon General, U. S. Army, and comparable *S. mansoni* material had been provided earlier by the School of Tropical Medicine, San Juan, Puerto Rico. To meet the immediate

practical need for information as to anticercarial treatment of water to be used for drinking and bathing, tests were made on mechanical removal of cercariae by filtration and on killing by chlorination. Preliminary studies were made on the effectiveness of chlorination of sewage in killing ova and miracidia. For evaluating measures for preventing skin penetration, chemically impregnated fabrics and ointments were tested. Laboratory colonies of the Philippine snail host, *Schistosomophora quadrasi*, were established and critical observations were made on environmental factors of significance in the breeding of these snails and of *Australorbis glabratus*. American snails which were regarded with suspicion as possible potential *S. japonicum* vectors were collected and identified, established as laboratory colonies, and tested as to their susceptibility to infection by tests similar to those already reported for *S. mansoni*. Various methods of preparing antigens of the two species of schistosomes were critically evaluated.

29. *The Making of a Parasitologist*. ASA C. CHANDLER. Rice Institute. Presidential Address.

30. *Sarcocystis in the Monkey. A Report of Two Cases*. EDWARD P. OFFUTT, JR. AND IRA R. TELFORD, University of Rochester and George Washington University.

In the course of the histological examination of tissue from six *Macacus rhesus* monkeys (Rochester colony) suffering from "cage paralysis," the presence in two of basophilic, granular, intramuscular-fiber inclusions was noted by one of us (I.R.T.). These structures were subsequently examined by the senior demonstrator and diagnosed as *Sarcocystis* sp.

It is not to be inferred that the "cage paralysis" necessarily resulted from the presence of the parasite. A further survey of this infection is now in progress.

The reported infrequent finding of this parasite in the monkey has led us to present this demonstration. Various sections of tissue, photographs, and charts pertaining to the infected animals have been made available for examination, along with certain other hosts parasitized by *Sarcocystis* sp. that have been encountered in the past in the laboratory at Rochester.

31. *Liver Coccidiosis Prevented by Sulfasuxidine*. H. A. JANKIEWICZ, College of Osteopathic Physicians and Surgeons, Los Angeles.

Eight weaned rabbits weighing from 3.5 to 5 pounds each consumed daily for 13 days about 0.625 grams of sulfasuxidine mixed in their feed. The average daily dose for the following 19 days was 0.75 grams. These rabbits each received 200,000 oöcysts of *Eimeria stiedae* on the day after the 0.75 gram dosage was instituted. Four controls not given sulfa were given a similar number of oöcysts; four others remained as non-infected controls. Eighteen days after the inoculation date, all rabbits were autopsied.

Grossly and microscopically the livers of the sulfa-fed rabbits appeared free from infection. No oöcysts were present in their gall bladders. However, the average liver of the sulfa-fed rabbit constituted 4.7% of the body weight compared to 3.7% for the uninoculated controls. The parenchymal cells appeared swollen and abnormal. Each inoculated control had a great number of typical biliary lesions and the average liver constituted 8.0% of the body weight.

The dosage of sulfasuxidine used did prevent the disease but appeared to be moderately toxic when given for a period of 32 consecutive days.

32. *A New Species of Opecoeloides (Trematoda: Opecoelidae) from the Threadfin Fish, Polynemus octonemus*. JANE HOGAN VON WICKLEN, University of Nebraska.

A new species of *Opecoeloides* is characterized by the peculiar arrangement of the ten acetabular papillae. Its study led to a revision of related species. Linton evidently placed too little significance on acetabular papillae in this group. Study of the type specimen of *Distomum vitellosum* Linton, 1900 shows that it belongs in the genus *Opecoeloides*; that it is different from *Cymbophallus vitellus* Linton, 1934 of Manter, 1934; and that *Opecoeloides manteri* (Hunninen and Cable, 1940) is one synonym. Various name changes are indicated in a paper now in press.

33. *Tumor Formation as a Reaction to Litomosoides carinii, a Filariid of the Cotton Rat*. J. ALLEN SCOTT AND JOY BARNES CROSS, University of Texas.

A wild cotton rat, *Sigmodon hispidus texianus*, presenting the appearances of advanced age, was killed three weeks after capture. The pleural cavity was found to contain only dead specimens of the filarial worm, *Litomosoides carinii*. In addition to worms lying free in the cavity, other dead worms extended through, or were coiled within three masses of tissue located in the superior mediastinum. Sections of these tumors show that the tissue reaction is primarily a typical inflammatory reaction with proliferation of connective tissue. The infiltration is composed for the most part of neutrophils which were scattered through the connective tissue, concentrated immediately around the worms, and to a slight extent invading the tissues of the worms.



Only occasional eosinophils are seen, and no multinucleate giant cells have been observed. About 15 per cent of the cells are histiocytes, both fixed and free, some of them containing nuclei of polymorphs. A large central area is filled primarily with disintegrating remnants of worms, worm eggs and a few polymorphs, but it does contain a few normal polymorphs and scattered connective tissue strands. Rarely small calcified areas are seen. Studies of related material from other animals indicate that these tumors are probably formed as a reaction to dead worms or possibly to worms which have been injured and have adhered to the tissues of the host.

34. *The Application of Cytological Techniques to Cestode and Other Helminth Material.* ARTHUR W. JONES AND HELEN L. WARD, University of Tennessee.

The chromosomes of cestodes can best be studied after fixation in suitable reagents, either in material sectioned at from 10 to 20 microns, or in flattened smear preparations. Sections may be stained with crystal violet, the Feulgen reagent, or iron haematoxylin. Smears may be stained with aceto-carmin or the Feulgen reagent. The chromosomes of trematodes and acanthocephala may be studied by similar methods.

35. *Preliminary Report on the Distribution of Onchocerca cervipedis.* CARLTON M. HERMAN AND ARTHUR I. BISCHOFF, California Division of Fish and Game.

This filariid nematode is a parasite of subcutaneous tissues of deer and possibly also occurs in prong-horn antelope and wapiti. In the past it has been reported primarily from western Montana. During the past two years the authors have examined one or more feet from about 330 deer from 16 counties in California. These included Rocky Mountain mule deer (*Odocoileus hemionus hemionus*), California mule deer (*Odocoileus hemionus californicus*), and black-tail deer (*Odocoileus hemionus columbianus*). Some specimens from all but three of these counties were positive. The worms apparently concentrate in greatest numbers under the skin about the hock joint of both fore and hind feet. Of 1220 feet examined, 447 were infected. Incidence varied from zero to 100 per cent in the deer from the areas sampled. In the three areas from which a large series was examined, incidence varied from 49 to 81 per cent. All worms collected were examined with the aid of a microscope. The worms were predominantly females. Examination of a number of entire fresh deer skins in the field revealed none of the worms although subsequent examination of the feet from some of these animals showed them to be infected. *Onchocerca cervipedis* has a wide distribution in deer in California. Examination of 200 feet from 52 prong-horn antelope (*Antilocarpa americana*) demonstrated no infection, although feet of deer from the same areas were positive.

36. *The Rate of Growth and Maturity of Litomosoides carinii, a Filariid of the Cotton Rat.* J. ALLEN SCOTT, University of Texas.

Experiments on the transmission of *Litomosoides carinii* have provided material for studies on the rate of growth and maturity of these filariids of the cotton rat. Worms as small as 870  $\mu$  have been found in the pleural cavity of the rats. Some of these smallest specimens have been recognized as males by the presence of a primordial cloaca. Females have been recognized by the presence of the vulva when as small as 2.0 mm. Since the infective larvae have been reported to vary from 800 to 1000  $\mu$  in length, it seems obvious that little or no growth takes place during the period of migration from the point of entrance into the definitive host to the final site. A moult from the fourth larval stage to the adult stage occurs when the worms of either sex are between 7 and 10 mm in length. Microfilariae were found in the circulating blood of rats 7 weeks old when they had been infected at an undetermined time, but presumably within a few days after birth. The male worms had sometimes completed half of their expected growth in length in rats 6 weeks old, while females reached this halfway mark in about 8 weeks. In wild-caught rats kept free from further infection for several months, the usual maximum length of male worms was 20 to 22 mm and of females 80 to 90 mm although a few females reached 100 mm. In the experimental rats some males reached 21 mm and some females 78 mm in as short a period as 10 weeks.

37. *Anatomical Studies on the Fourth Stage Larvae and Adults of Litomosoides carinii, a Filariid of the Cotton Rat.* JOY BARNES CROSS AND J. ALLEN SCOTT, University of Texas.

Out of 2000 available specimens of *Litomosoides carinii* from the Texas cotton rat, *Sigmodon hispidus texianus*, 200 have been selected for intensive study. Descriptions of the developmental patterns of the cuticular structures, the somatic musculature, and the digestive and reproductive systems are presented. The worms varied in length from about 1 mm to maximum sizes of 22 mm for the male and 100 mm for the female. A moult from the fourth larval stage to the adult stage occurs when the worms are between 7 and 10 mm in length. After removal from the host the mature females can be stimulated to discharge in some cases oviform and in others vermiform



larvae, while still others discharge both types. Artificially produced empty spaces have thus been left in the uterus. Moreover, the vaginae of the worms have been observed to contain one type of larvae or the other exclusively, or both types together. These variations and others seen in the normal histological picture point to the need for extreme care in interpreting histological changes occurring after the worms have been subjected to various drugs, and for a knowledge of the histological variation which can result from differences in methods of handling the worms at autopsy. In addition, there is commonly a great difference in the degree of development of the two branches of the ovary or uterus at the same level in the worm, as well as similar variations between homologous regions in different worms of the same length.

38. *Treatment of Canine Filariasis with Trivalent Arsenicals (p-arsenosobenzamides)*. G. F. OTTO AND THOMAS H. MAREN, Johns Hopkins University.

Preliminary studies with microfilaria of *Dirofilaria immitis* showed that the phenyl arsenoxides were 10 to 400 times as toxic as the trivalent antimonials for these organisms *in vitro*. Subsequent studies on cotton rats showed that of these phenyl arsenoxides the p-amide substituted compounds regularly killed all adult *Litomosoides carinii* and were well tolerated by the host. Of these amides p-arsenosobenzamide (T.D.C. #622) itself was extensively studied in the dog and was shown to kill all the adults of *D. immitis* in doses as low as 0.45 mgm As/K daily for six weeks. Because of the low solubility in water, work with this compound was discontinued and one of its more soluble thio-arsenites,  $p\text{-CONH}_2\text{C}_6\text{H}_4\text{As}(\text{SCH}_2\text{COOH})_2$ , was synthesized.

The neutral sodium salt of this compound has been shown, to date, to kill all adults of *D. immitis* in doses of 0.45 mgm As/K daily for 15 days. Neither p-arsenosobenzamide or the thio-arsenite seems to have any direct killing action on the microfilaria *in vivo* in doses of 0.9 mgm As/K for 30 days despite the fact that *in vitro* all microfilaria were killed in 22 hours in dilutions as low as 1:800,000 (in terms of arsenic). Apparently after the death of the adult worms the microfilaria die slowly without appreciable ill effects from the arsenic. One dog treated with 0.45 mgm As/K daily for 50 days maintained an undiminished microfilaria blood level for 100 days thereafter; now, 200 days after the end of treatment, the microfilaria count has dropped to less than one-fifth of the pretreatment level. (This work was done under contract with the Office of Scientific Research and Development.)

39. *The Treatment of Human Filariasis (Wuchereria bancrofti) by Administration of Melarsen Oxide*. HARRY M. ROSE AND JAMES T. CULBERTSON, Columbia University.

During April and May 1945 eighteen male patients with Bancroftian filariasis were treated in Puerto Rico with a new trivalent arsenical compound, melarsen oxide. None of these patients exhibited symptoms of the infection, but all showed microfilariae in night blood specimens.

Three patients received the drug orally, 50 mgm three times daily, for seven to fourteen days. Seven individuals were given the drug, dissolved in propylene glycol, intravenously in doses of 7.5 mgm daily for seven consecutive days. The remaining eight patients, received 10 mgm intravenously daily for eight or nine consecutive days.

In all cases control microfilarial counts were done before treatment was begun and at intervals thereafter. Six to seven months after treatment microfilariae had completely disappeared from the blood of 6 patients, while the counts were markedly reduced in 5 cases, moderately reduced in 4 cases, and unchanged in 3 cases. The most favorable results were obtained in the group of patients that were given the 10 mgm doses of melarsen oxide intravenously.

Severe reactions, characterized by toxic encephalopathy, were observed in two instances, with complete recovery in both. These reactions indicate that the drug must be administered with caution, but do not detract from the observation that arsenical compounds of this class, the arsenoxides, may be of value in the therapy of human filarial infections. (This work was done under contract with the Office of Scientific Research and Development.)

40. *Studies of Sheep Parasites. VI. Observations on Weather in Relation to Untreated Nematode Infections*. PHILIP A. HAWKINS, Michigan State College.

Studies of egg counts of sheep over a three-year period have shown that infections with strongyles and trichostrongyles are directly proportional to moisture and temperature. Ewes become reinfected with the first onset of warm weather in the spring. They undergo a light infection, and then are capable of throwing off most of this and maintaining a low-grade infection during the summer. If excessive rainfall occurs during the summer, the resistance of the animal is broken down and heavy reinfection occurs. In the lamb the same picture is observed except that the infection is much more severe and occurs three to four months later. In the lamb the infection is less severe and of shorter duration in dry summers, whereas in summers with excessive precipitation, heavy infection with accompanying high mortality occurs.

41. *Immunity to Necator americanus Infection.* PAUL C. BEAVER, Tulane University.

An experimental infection of three months duration failed to influence appreciably the development of subsequent infections of *Necator americanus* in a human subject. Although the severity of cutaneous reaction to the penetration of larvae was increased with each succeeding exposure, the intestinal infection as measured by output of eggs was proportionate to the number of larvae used. The initial exposure to 200 larvae produced counts of 4,000 to 5,000 eggs per cc (f.s.b.) and additional exposures to 800 larvae increased the counts to above 20,000. Attempts to obtain precipitin reaction to living filariform larvae of *N. americanus* in the subject's serum were consistently negative after 18 months of continuous infection.

42. *Infection Experiments with a Hookworm of the Cotton Rat.* LYELL J. THOMAS, University of Illinois.

Infective filariform larvae were obtained from fecal cultures which contained a natural infection of hookworm eggs from the cotton rat. Although nearly 150 of these larvae were applied to the left forearm of the author and penetration was observed under the microscope no creeping eruption or intestinal infection occurred.

Cotton rats were easily infected by giving them larvae in their drinking water. The rats could not be infected with *Necator americanus*.

43. *Hydrogen Ion Concentration as a Factor in Age Resistance to the Fowl Ascarid.* B. B. RIEDEL AND J. E. ACKERT, Kansas State College.

It is known that young chickens kept on an adequate diet will develop, as they grow older, increased resistance, or age resistance to the growth of the fowl nematode *Ascaridia galli*. Various factors in the increased resistance have been determined and evidence has been presented which indicates that the limiting factor is given off from the wall of the small intestine into the lumen where the young ascarids live. The duodenal mucus of this portion of the intestine appears to contain a resisting factor. This substance is secreted by the goblet cells. However, the possibility remained that variations in the hydrogen ion concentration might be an important factor in the resistance.

Two groups of single comb white leghorns of sixty chicks each were used to make the study. Twenty-one days before autopsy each chicken of both groups was given 500 *Ascaridia galli* eggs. The first group of chickens was killed on the 28th day, while the second group was killed on the 93rd day after hatching. Comparisons between the two groups of chickens were made on the basis of worm number, worm length and hydrogen ion concentration.

The four-week-old chicks had an average of 11.8 worms, while the thirteen-week-old fowls had an average of 2.3 worms. The average length of the worms of the four-week group was 23.3 mm, while that of the older group was 20.6 mm. The hydrogen ion concentration of the worm habitat in the four-week-old chicks was 6.50 as compared with 6.34 for the thirteen-week-old chickens—but a slight difference.

These results which show a nearly constant hydrogen ion concentration for chickens from four to thirteen weeks of age, indicate that variation in hydrogen ion concentration is not a factor in age resistance of chickens to ascarids.

44. *An Anomalous Experience with Phenothiazine.* PAUL D. HARWOOD, Dr. Hess and Clark, Inc., Ashland, Ohio.

In general the larger animal is given the larger dose of an anthelmintic to maintain approximately the same lethal concentration of drug in the intestines of small and large hosts. However, experience with more than 200 chickens suggests that the effective dose of phenothiazine against *Heterakis gallinae* is lower for heavy birds than for light birds. Thus 0.44 grams of phenothiazine per bird removed 89.8 per cent of 675 *Heterakis* from 12 Brown Leghorn cockerels averaging 1208 grams; 0.5 grams removed 95.7 per cent of 1854 *Heterakis* from 18 Ancona cockerels averaging 1847 grams; 0.35 gram removed 99.3 per cent of 1083 *Heterakis* from 17 White Rock cockerels averaging 2004 grams; and 0.1 gram of phenothiazine removed 91.3 per cent of 987 *Heterakis* from 32 White Rock cockerels averaging 3048 grams. Remaining data may not be presented in an abstract. Since these experiments were not designed primarily to test the effect of size of host on anthelmintic medication, the observed results may be due to some factor other than size of host at time of treatment, such as variety. However, very limited tests with phenothiazine against *Heterakis* in turkeys and pheasants suggests that the drug is very effective in the former host but of doubtful value in the latter. These data are presented at this time largely to raise the question of the true relationship between size of dose and size of host.

45. *Studies on the Cytochrome Oxidase of the Pig Ascaris.* C. A. HERRICK AND MARIE THEDE, University of Wisconsin.

The oxygen uptake of pig ascaris homogenate was determined by means of the Warburg

apparatus using the Potter technique. The oxygen uptake of the homogenate alone was negligible but was slightly increased by the addition of sodium succinate. When cytochrome c was added to the tissue-succinate mixture it was immediately and permanently reduced and the respiration of the tissue was not increased.

When the oxygen carrier, brilliant cresyl blue, was added to the homogenate plus sodium succinate it greatly increased the oxygen uptake of the tissue. It was, however, ineffective in the absence of this substrate.

The use of ascorbic acid as a substrate to furnish an excess of reduced cytochrome c was ineffective in increasing the tissue respiration. This, together with the fact that when diethylstilbestrol was added to the tissue-succinate-cytochrome mixture, respiration was not reduced, indicates that cytochrome oxidase is lacking in the adult pig ascaris.

46. *Additional Studies on the Effects of Nodular Worm Infections on Calves During the Prepatent Period.* ROY L. MAYHEW, Louisiana State University.

The results obtained in experiments in which five calves were inoculated with pure cultures of nodular worm larvae indicate that very severe symptoms of parasitosis develop during the prepatent period of the infection. There is loss in weight, the animals refuse to eat and drink, and at post-mortem much inflammation is evident in the small and large intestine. These symptoms are generally believed in natural infections to be associated with damage caused by the adult parasites. Calves that survive the prepatent period gain in weight and strength during the period of greatest egg-count in the adult period of the life of the parasite. Such evidence suggests that the improvement following the use of anthelmintics, when severe parasitism is diagnosed, is actually a natural comeback following the effects that were produced by the larvae during the prepatent period rather than by elimination of the adults. These findings confirm those reported in a previous publication (Cornell Vet. 34: 299-307) giving the results of pure stomach worm and mixed stomach worm and nodular worm inoculations.

47. *The Relationship in Mice of Intestinal Emptying Time and Natural Resistance to Hymenolepis.* JOHN E. LARSH, JR., University of North Carolina.

Mice have a strong natural resistance to *H. nana* var. *fraterna* as shown by low percentages of cysticeroid development (usually much less than five) following initial infection. The explanation for this is unknown, but since large numbers of viable eggs pass unchanged through the intestine, it has been suggested that emptying time may be involved. To obtain an idea of the elimination rate, a group of mice was given carbon ink solution by mouth. Periodic autopsies revealed large amounts of the ink in the lower colon within 15-20 minutes. If eggs are eliminated similarly, it seems plausible that many would be excreted before sufficient time elapsed for hatching. If this is the case, then slowing the rate of elimination should result in higher percentages of cysticeroid development. In order to slow intestinal passage, drug action has been the most successful method used thus far. Opium given parenterally in various concentrations retarded the passage of ink from one to several hours. Moreover, when given just prior to infection, this drug in test animals increased the percentage development of cysticeroids many times above that of controls. While opium may have more than a single effect, it is highly suggestive that the increased percentage development of cysticeroids in mice given the drug was due to retarded passage of the eggs.

48. *Cercarial Production in Snails and Metacercarial Infections in Fish from Carrol Lake, Wisconsin.* L. O. NOLF, University of Wisconsin, Wisconsin Conservation Department, and University of Iowa.

During the summer of 1944, 2776 snails were isolated to get emerging cercaria. Seven hundred sixty of these produced cercaria. In 1945, 686 snails out of 2773 shed cercaria. The cercarial production was low during May with a sharp rise in June reaching a peak early in July followed by a slight decline through August. The highest percentage of infection with furcocercous cercaria was found in *Stagnicola exilis*. The second highest was in *Physella sayii*. While *Heliosoma trivolvis* was third in the percentage of snails that shed furcocercous cercaria, because of their greater abundance, they produced nearly half of the total infections of this type. During the summer of 1945, in collaboration with F. G. Brooks, we found a number of Xiphidio-cercaria that readily penetrated fish, some with lethal effect. The incidence of infection in snails varied at different stations. The percentage of infected snails remained quite constant for the two seasons.

A pepsin-digest technique was used to make a quantitative study of the metacercarial infections in fish. The numbers of metacercaria recovered from fish ranged from one to 90,250. The highest was from an experimentally infected fish. This was an intensity of infection of 1612 metacercaria per gram weight which is about four times as great as the highest natural infection found, namely, 20,600 or 412 metacercaria per gram weight.



Blue gills had more than twice as many metacercariae per gram body weight as did the Perch. This difference is even greater in the zero age group. Differences in degree of infection was noted in fish from different stations.

49. *Ecology of the Metacercariae of Fasciola hepatica in Southern Texas and Its Relationship to Liver Fluke Control in Cattle.* O. WILFORD OLSEN, U. S. Bureau of Animal Industry.

In southern Texas, where the snail intermediate host (*Stagnicola bulimoides techella* (Hald.)) of the liver fluke lives primarily in temporary pools, the cercariae are able to encyst only when standing water is present on the pastures which is usually during the winter and early spring.

Post-mortem examinations and field studies indicated that both larval and adult flukes were present in cattle during the early summer but primarily only adult flukes were found in the late fall. This condition suggested that the pastures became free from infective metacercariae during the summer. In order to test the viability of the metacercariae during the later summer and fall of 1944, one fluke-free sheep was placed on each of 2 pastures on the first of each month, beginning with September and extending through March. Fecal examinations were made at monthly intervals to determine when the sheep became infected.

Fluke eggs first appeared February 28, 1945. Allowing 3 months for the flukes to mature following ingestion of the metacercariae, it appeared the pasture was free from viable cysts from September 1 to December 1. How much before September the metacercariae were killed by the heat and drought was not ascertained.

Since larval flukes migrating in the liver parenchyma are not killed by hexachlorethane and young flukes in the bile ducts are more resistant than adults (Olsen, 1944, J. Parasitol. Suppl. 30: 14), medication is most effective late in the fall when the flukes are mature. Moreover, medication in the late fall greatly reduces the number of fluke eggs passing on to the pastures when moisture conditions are favorable for their hatching and infecting the snails.

50. *Determination of the Life History of Cercaria szidati, a Furcocercous Larval Trematode of the Vivax Type.* DORCAS J. ANDERSON, Purdue University.

*Cercaria szidati* Anderson, 1944, penetrates minnows and encysts in the muscles, especially of the tail region. Heavily infected minnows were fed to albino rats, mallard ducklings, and several series of newly hatched chicks. In only a single series of five chicks did the trematodes develop to maturity; two yielded 14 and 30 mature worms, respectively, when killed and examined three days after receiving metacercariae and the remaining three chicks passed eggs but threw off the infection in less than a week. A Great Blue Heron nestling acquired a heavy infection but the egg output fell off rapidly and subsequent massive doses of metacercariae failed to become established. The adult is an undescribed species of *Linstowiella* and the fact that both it and the cercarial stage are monostomatous is of interest since closely related species are typical holostomes. The embryological development of the complex cercarial excretory system has been investigated.

51. *Motion Picture of Cercaria clausii Monticelli, a Marine Rattenkönig Larval Trematode from the West Coast of Florida.* R. M. CABLE, Purdue University, AND RICHARD A. MCLEAN, Academy of Natural Sciences of Philadelphia.

A short motion picture of *C. clausii* illustrates the behavior of this unusual type of larval trematode. In some sequences, the clusters of larvae move as units, all members contracting simultaneously, while in others, the individual cercariae move independently as if attempting to detach themselves from the rosette.

52. *Schistosomiasis.* PAUL P. WEINSTEIN, U. S. Public Health Service.

The salient features in the life cycle, geographical distribution, epidemiology, symptomatology, pathology, diagnosis, and methods of treatment of the three types of schistosome infections of man are presented in a colored film strip. It represents the combined efforts of a number of individuals to portray in an attractive and interesting manner the known authoritative information on these diseases. This film strip with an accompanying recorded narration is now being made available to all interested teaching institutions and educational groups by the Training and Education Division of the United States Public Health Service, 291 Peachtree Street, Atlanta, Georgia.

53. *The Laboratory Diagnosis of Schistosomiasis.* M. M. BROOKE, U. S. Public Health Service.

This film strip of colored photographs demonstrates the step by step procedures of making laboratory diagnosis of schistosome infections. The examination of fecal samples and urine



specimens include the simple smear, sedimentation, centrifugation, acid-ether, and hatching techniques. For routine concentration purposes, a modified sedimentation and centrifugation technique is recommended. For small samples, and where expense is not an item, the acid-ether test is considered preferable. This film strip has been prepared for use in the courses on "The Laboratory Diagnosis of Parasitic Diseases," currently being offered by the Diagnostic and Training Laboratory of the United States Public Health Service in Atlanta, Georgia. Accompanied by a recorded narration, this strip and others, now in production, will also be made available to teaching institutions and professional groups that request them.

54. *Comparison of Methods for Diagnosing Schistosomiasis japonica by Recovery of Eggs from Feces.* GEORGE W. HUNTER, III, JAMES W. INGALLS, AND MINNA G. COHEN, Army Medical School.

The following methods for recovering eggs of *S. japonicum* from dog and human stools were tested semiquantitatively: direct smears, sedimentation, sugar flotation, zinc sulfate flotation, zinc sulfate-centrifugation-flotation, acid-ether, and acid-Triton NE-ether. Both the acid-ether and acid-Triton NE-ether proved to be more efficacious than the other methods which were tested.

Routine laboratory tests using direct smears, sedimentation, and acid-Triton NE-ether yielded superior results with the latter technique. A modification of the acid-Triton NE-ether method consisting of 2.5 cc of hydrochloric acid, 2.5 cc of sodium sulfate, 0.06 cc of Triton NE, and 5 cc of ether was tested on dog and human stools and compared with the acid-Triton NE-ether, sedimentation and direct smear methods. This new modification of the acid-Triton NE-ether method appears superior in a limited series of tests to other techniques commonly employed for the recovery of *S. japonicum* eggs.

55. *Periodicity of Microfilariae in the Philippines.* J. L. AVERY, Naval Medical School.

*Wuchereria bancrofti* exhibits two distinct strains based on the presence or absence of periodicity: periodic, in which microfilariae are found in the peripheral circulation only at night; and non-periodic, in which circulating microfilariae may be found in uniform abundance at any time. The geographical distribution of these two strains is well determined, with no overlapping. However, in the strain of *W. bancrofti* encountered in natives of the central Philippine archipelago, small numbers of circulating microfilariae could be demonstrated during the daylight hours and relatively much greater numbers at night. Results of our surveys support the contention of Philippine workers that this condition represents a modified nocturnal type of periodicity rather than either one of the established strains or an overlapping of the two types.

56. *The Thermal Death Point of Cysticercus bovis.* REX W. ALLEN, U. S. Bureau of Animal Industry.

Controlled tests to determine the thermal death point of *Cysticercus bovis* were conducted by using 439 cysticerci from four naturally infected host animals. The viability tests used were (1) ability to evaginate in 0.6 per cent sodium taurocholate solution, (2) flame cell motion, and (3) ability to pass undigested through the digestive tract of a human subject.

A total of 192 decapsulated cysticerci were heated gradually in physiologic saline to temperatures of 50°, 53°, 54°, 55° and 56° C. The time required to reach these temperatures ranged from 3 1/3 to 5 1/2 minutes. Viability tests showed evidence of life in cysticerci heated to temperatures as high as 55° C, but showed none in the 132 cysticerci heated to 56° C.

A total of 124 cysticerci were recovered from small pieces of host muscle tissue heated gradually in physiologic saline to internal temperatures of 45°, 50°, 55°, 56° and 57° C. The time required to reach these temperatures varied from 13 1/2 to 30 minutes. It was found that an internal temperature of at least 56° C in the pieces of flesh was necessary in order to kill all the contained parasites. Eight pieces were heated to 56° C, and they were found to contain 46 cysticerci all of which failed to show evidence of life when subjected to the viability tests.

57. *The Nature of the Mechanism of Encapsulation in Trichiniasis.* WAYNE W. WANTLAND, CORRINE L. BARDES, AND ROBERT S. LEVINE, Illinois Wesleyan University.

Following entrance of trichina larvae into the striated musculature of the host, inflammation is set up by the movements and waste products of the rapidly growing larvae, the fiber becomes granular and swells, giving rise to pressure necrosis and degeneration of the surrounding fibers. Considerable white-cell infiltration occurs and the fibers of the interfascicular connective tissue undergo hypertrophy. A delicate parietal layer is then formed around each larva, which is apparently the beginning of a fibrosis resulting from the alternation of the normal condition of the tissues. The infected tissue undergoes rapid histological changes and an intercellular exudate is formed. The delicate parietal layer surrounding the invading parasite arises by a direct and gradual transformation of the minute elements of the exudate. This transformation is

effected by the pressure and tension which is applied to the fibrillations that appear in the exudate, as a result of inflammation and swelling of the invaded fiber. The enveloping sheath becomes thicker as more fibrillated layers are added. The wall becomes homogeneous and hyaline in nature and its deeper layers unite at the poles of the cyst. In some instances complete atrophy of the muscle fiber results; at other times the striation appears normal above and below the cyst and the sarcolemma is continuous with the most external layer of the cyst wall. The fibrous nature of the cyst can be readily seen in both fresh and fixed preparations of infected rat and rabbit diaphragm muscle when viewed directly with the microscope. Photomicrographs of such preparations show the hyaline nature of the capsule.

58. *On the Distribution of Glycogen in the Trematode, Ostiolum sp.* JAMES H. WILMOTH AND RHODA GOLDFISCHER, Brooklyn College.

The distribution of complex polysaccharides (i.e., glycogen) has been investigated in *Ostiolum sp.*, a parasite of *Rana pipiens*, using histological and histochemical techniques. Best's carmine method, Lugol's solution, and the Feulgen-Bauer reaction were used to demonstrate the location of glycogen. Most reliance was placed on the latter technique. Control tests were conducted on *Ostiolum* and on mouse liver. The paraffin method was employed throughout and complete serial sections of the trematode were used. The presence of specific staining granules in muscle tissue, eggs, and parenchyma of this parasite indicates that glycogen deposits are present in these locations. However, similar granules were not observed in the cuticle, excretory ducts, seminal receptacle, testis, and vitellaria and, therefore, glycogen is considered to be absent from these portions.

Some preliminary studies, employing vital-staining and osmic methods, were made on the structure and distribution of Golgi bodies in this trematode.

59. *Studies on the Respiration of the Frog Lung Fluke, Ostiolum sp.* JAMES H. WILMOTH AND NORA LEVITAS, Brooklyn College.

Respiration studies on *Ostiolum sp.* from the lungs of *Rana pipiens*, indicate that this trematode utilizes oxygen from the environment. Varying numbers of worms were placed in Frog Ringer solution in a closed system and the Ringer was tested at regular intervals for oxygen content by the Winkler method. Parasites freshly removed from the host as well as those maintained *in vitro* for periods of time up to five days utilized the oxygen present in the Ringer at much the same rate. Worms stained in a dilute solution of methylene blue and placed in sealed depression slide chambers were able to reduce the methylene blue when free oxygen was no longer present in the environment. Worms maintained *in vitro* for over seven days were motile under anaerobic as well as aerobic conditions.

60. *A Note on the Genus Axine Abildgaard (Trematoda; Monogenea).* EMMETT W. PRICE, U. S. Bureau of Animal Industry.

The body outline of species of the genus *Axine* is in general that of an obtuse triangle, the shorter leg of the triangle representing the haptor part of the body. Haptor hooks have not been reported for species of the genus *Axine* (*s.str.*). An examination of several species, including the genotype, *Axine bellones*, revealed a small languette or lobe bearing 2 pairs of minute hooks. This lobe is situated along the row of haptor clamps at a point corresponding to the distal end of a line drawn through the long, medial axis of the body. Correlated with the presence of this lobe are a U-shaped ovary, an armed vagina and, usually, a more or less definitive pattern of genital spines. Only species having this combination of characters should be included in the subfamily Axininae.

Three genera of Axininae are recognized: (1) *Axine* Abildgaard (Syn. *Cestracolpa* Meserve) (vaginal opening lateral, with horn-like spine), including *A. bellones* Abildgaard (type), *A. cypseluri* (Meserve), *A. gracilis* Linton, *A. inada* Ishii and Sarwada, *A. japonicum*, n.n. for *A. cypseluri* Yamaguti, *A. yamagutii* (Meserve), and *A. triglae* Beneden and Hesse; (2) *Axinoides* Yamaguti (vaginal opening median, with horn-like spine), including *A. aberrans* Goto, *A. meservei* for *A. aberrans* of Meserve, and *A. tylosuri* Yamaguti (type); and (3) *Neoaaxine*, n.g. (vaginal opening lateral, with incomplete circle of spines), type and only species *N. constricta* (Yamaguti).

All other species at present in the genus *Axine* will be placed in a new subfamily.

61. *Additional Observations on the Life History of Gorgodera amplicava Looss, 1899.* CHAUNCEY G. GOODCHILD, Southwest Missouri State College.

A restudy of the life history of *Gorgodera amplicava* from Cape Cod, Massachusetts, has been made. *Musculium partumeium*, molluscan host of *G. amplicava*, collected from the same ponds as *Musculium securis* and *Sphaerium occidentale*, has been found to be the only natural

host. The large-tailed cercaria of *G. amplicava* agrees closely in most details with published descriptions, but has been found to be much longer than previously reported. A length of 17.4 mm (12 mm–17.4 mm; average about 13.9 mm) has been determined for recently shed cercariae. *Physa* sp. and several species of tadpoles, including those of *Rana catesbeiana*, *R. palustris*, *R. clamitans* and *Hyla versicolor* are natural metacercarial hosts while *Ambystoma* sp. has been experimentally infected with these cercariae. Penetration and early encystment of metacercariae have been observed in laboratory fed odonatan larvae (*Enallagma* sp.). The vertebrates in which metacercarial excystment occurs with subsequent orientation of the young worms to the excretory ducts are: *Rana catesbeiana*, *R. palustris*, *R. clamitans* and *R. pipiens*. Excysted worms have been recovered from the oviducts of *Ambystoma* sp. Normal metacercarial excystment takes place in the gut of *Triturus viridescens viridescens*, but the worms become quickly trapped in mucus covered fecal pellets and are eliminated. This fact may be a possible explanation of the "immunity" of certain amphibian species to gorgoderid trematodes. The miracidia, approximately 55  $\mu$  long by 82  $\mu$  wide, are covered with fifteen epidermal plates arranged in three rows. The pattern is 6, 6, 3. The free-swimming miracidia are drawn, with incurrent water, into the bivalve host where they penetrate the gills and transform into mother sporocysts.

62. *Studies on Parasites of Bassalian Fishes.* ROSS F. NIGRELLI, New York Aquarium and New York Zoological Society.

Little is known about the parasites of bathybial and semi-bathybial fishes. Through the courtesy of Dr. William Beebe (New York Zoological Society) several species of these rare forms were made available for study. In this preliminary survey the following species were examined: *Cyclothones* spp. (100–1000 fathoms), *Lampanyctus cuparius* Taning (50–1000 fathoms), and *Echiostoma tanneri* (Gill) (500–900 fathoms). *E. tanneri* was the only form found infected. Larval nematodes of a single species belonging to the subfamily Anisakinae were found encysted in the body cavity. No special modifications were present in the parasite that could be correlated with either its host or its environment. The parasite belongs to that group of ascarids characterized by the presence of an oesophageal appendix and absence of an intestinal caecum. The worms measure from 7–25 mm in length, and from 0.4–0.6 mm in width (at the region of the vulva). The oesophagus varies in length from 1.2–2.9 mm and the ventriculus from 0.5–0.7 mm. The appendix is comparatively long, varying from 2.4–3.8 mm. Typical trilobed larval lip structures and boring tooth are present. The posterior end terminates in a retractile spine-like structure. The anus is situated from 0.5–0.7 mm from the tip. Giant excretory (?) cells are present in the region of the terminal part of the intestine. It would be difficult to state what the final hosts of these parasites are, or how the fish become infected at these extreme depths since nothing is known about their predators or what they eat.

63. *Parasite Studies of Quail, Colinus virginianus and Colinus virginianus texanus, in Mississippi.* J. W. WARD, Mississippi State College.

A total of 273 Bobwhite quail, *Colinus virginianus* and ten Mexican quail, *Colinus virginianus texanus*, collected in Mississippi have been examined for internal parasites. The collection of material extended over a period of five years. A comparison of infestations in the ten soil areas of Mississippi has been made. It was shown that parasitism reached the highest level in 1941–42. Those birds collected from the Brown Loam and Bluff areas were the most heavily parasitized. Eleven species of internal parasites were found. They were distributed as follows: seven nematodes, *Heterakis gallinae* (Gmelin, 1790) Freeborn, 1923; *Heterakis bonasae*, Cram, 1927; *Subulura brumpti* (Lopez Neyra, 1922) Cram, 1926; *Seurocyrnea colini* Cram, 1927; *Habronema pileata* Walton, 1927; *Syngamus trachea* (Montague, 1811) Chapin, 1925; *Trichostrongylus pergracilis* (Cobbold, 1873) Railliet and Henry, 1909; three cestodes, *Raillietina* (*Skrjabinia*) *cesticillus* (Molin, 1838) Joyeux, 1923; *Hymenolepis carioca* (Magalhaes, 1898) Ransom, 1902; *Rhabdometra odiosa* (Leidy, 1887) Jones 1929 and one or more coccidia, *Eimeria* spp.

A key has been prepared to be used in identifying the known species of helminth parasites of quail.

64. *The Nematode Parasites of the Bufoninae (Amphibia—Salientia—Procoela—Bufonidae)* I. A. C. WALTON, Knox College.

The annotated list of the Nematode parasites of the Bufoninae indicates the following hosts and parasites: 1a. *BUFO AMERICANUS* (Brazil, Zool. Gard.)—*Oswaldocrusia subauricularis*. 1b. (U.S.A.)—*Hedruris* sp.?, *Oswaldocrusia* ?*filiformis*, *O. leidy*, *O. pipiens*, "*Oxyuris*" *dubia*, and *Rhabdias bufonis*. 2. *B. BOREAS* (U.S.A.)—*Cosmocercoides dukae*, *Oswaldocrusia waltoni*, and *Spironoura pretiosa*. 3. *B. BUFO* (Europe)—*Aplectana acuminata*, *Cosmocerca commutata*, *C. ornata*, "*Filaria*" *jubae*, *Oswaldocrusia filiformis*, *Oxysomatium brevicaudatum*, *O. contortum*,



*O. longispiculum*, *O. praeputiale*, "*Oxyuris*" *mucronata*, "*O.*" *tarda*, *Rhabdias bufonis*, *R. microoris*, *R. rubrovenosa*, and *R. sphaerocephala*. 4. B. B. ASIATICUS (China)—*Africana howardi*, larval *Habronema mansonii*, and *Rhabdias bicornis*. 5a. B. B. FORMOSUS (China)—*Africana howardi*. 5b. (Japan)—*Capillaria bufonis*, and *Oswaldocruzia filiformis* (= *O. insulae*). 6. B. B. GARGARIZANS (China)—*Oswaldocruzia peipingensis*. 7a. B. B. JAPONICUS (China)—*Cosmocercoides pulcher*, and *Spinicauda japonica*. 7b. (Japan)—*Capillaria bufonis*, *Cosmocercoides pulcher*, *Oswaldocruzia bialata*, *Rhabdias incerta*, and *Spinicauda japonica*. 8. B. CALAMITA (Europe)—*Hedruris androphora*. 9. B. CANORUS (U.S.A.)—*Cosmocercoides dukae*. 10. B. COGNATUS (U.S.A.)—larval Physalopterans. 11. B. COMPACTILIS (U.S.A.)—larval Physalopterans. 12a. B. CRUCIFER (Brazil)—*Aplectana crucifera*, *Cosmocerca brasiliensis*, *Oswaldocruzia subauricularis*, *Oxyascaris similis*, *Oxysomatium membranosa*, and *Schulzia subventricosa*. 12b. (Zool. Gard., Europe)—*Oswaldocruzia filiformis*. 13. B. FOWLERI (U.S.A.)—*Cosmocercoides dukae*, *Oswaldocruzia pipiens*, *O. waltoni*, Physalopteran larvae, *Rhabdias ?bufonis*, *R. ranæ*, and *R. sp.?* 14. B. HIMALAYANUM (India)—*Cosmocercoides bufonis*.

65. *The Nematode Parasites of the Bufoninae. II.* A. C. WALTON, Knox College.

15a. BUFO MARINUS (Brazil)—*Ankylostoma* sp.?, *Aplectana acuminata*, *A. vellardi*, *Cosmocerca commutata*, "*Filaria*" larvae, *Foleyella vellardi*, *Oswaldocruzia mazzai*, *O. subauricularis*, *Oxyascaris similis*, *Oxysomatium membranosa*, and *Rhabdias fülleborni*. 15b. (Zool. Gard., England)—"*Filaria*" larvae, Microfiliariids, and *Rhabdias bufonis*. 15c. (Fr. Guiana)—"*Filaria*" sp.? 15d. (Mexico)—*Aplectana hoffmanni*, *Cruzia morleyi*, *Icosiella* sp.?, *Ochoterenella digiticauda*, *Oswaldocruzia subauricularis*, and *Rhabdias sphaerocephala*. 15e. (Panama)—"*Filaria*" sp.? 15f. (Puerto Rico)—*Ascaris lumbricoides* eggs, and *Trichuris trichiura* eggs. 16. B. MAURITANICUS (Africa)—*Aplectana* sp.?, *Oxysomatium macintoshii*, and *Spirocerca lupi*. 17a. B. MELANOSTICTUS (Burma)—*Meteterakis govindi*, *Oswaldocruzia filiformis*, and *Oxysomatium macintoshii*. 17b. (China)—*Meteterakis govindi*, *Oswaldocruzia hepatica*, *O. hoepplii*, *Oxysomatium macintoshii*, *Rhabdias bufonis*, and *Spirocerca pectinospiculata*. 17c. (Formosa)—*Spinicauda bufonis*. 17d. (Fr. Indo-China)—*Oswaldocruzia filiformis*, *O. hoepplii*, and *Oxysomatium macintoshii*. 17e. (Siam)—*Rhabdias brachylaimus*, and *Spirocerca* sp.? 18. B. PELTOCEPHALUS (Cuba)—*Aplectana hamatospicula*, and *Strongyloides amphibiophilus*. 19. B. QUERCICUS (U.S.A.)—*Cosmocercoides dukae*, and *Oswaldocruzia pipiens*. 20. B. REGULARIS (Cent. Africa)—*Africana africana*, *Amplicaeum involutum*, "*Filaria*" (2 spp.?), *Foleyella leiperi* (and larvae), and *Oxysomatium praeputiale*. 21. B. SIMUS (Mexico)—*Aplectana mexicana*. 22. B. STEINDACHNERII (Africa)—Nematodes. 23. B. STOMATICUS (India)—*Oxysomatium macintoshii*, and larval Physalopterans. 24. B. TERRESTRIS (U.S.A.)—*Cosmocercoides dukae*, *Oswaldocruzia leidyi*, *O. pipiens*, *Oxysomatium americana*, and *Rhabdias ranæ*. 25. B. VALLICEPS (U.S.A.)—*Aplectana* sp.?, *Cosmocercoides dukae*, and *Oswaldocruzia pipiens*.

66. *The Nematode Parasites of the Bufoninae. III.* A. C. WALTON, Knox College.

26a. BUFO VIRIDIS (Africa)—*Oxysomatium macintoshii*. 26b. (Europe)—*Agamospirura* sp.?, *Aplectana acuminata*, *A. brumpti*, *Atractis* sp.?, *Cosmocerca commutata*, *C. ornata*, "*Filaria*" *parva*, "*F.*" *unguiculata*, *Oswaldocruzia filiformis*, *Oxysomatium brevicaudatum*, *O. linstowi*, *O. longispiculum*, *O. macintoshii*, "*Oxyuris*" *tarda*, "*O.*" *iba*, *Rhabdias bufonis*, *R. microoris*, *R. rotundata*, and *R. rubrovenosa*. 27. B. WOODHOUSEI (U.S.A.)—*Abbreviata ranæ*, and *Oswaldocruzia pipiens*. 28. BUFO spp.? (Argentina)—*Oswaldocruzia mazzai*, and *O. subauricularis*. 29. BUFO spp.? (Europe)—"*Filaria*" larvae, and *Oswaldocruzia filiformis*. 30. NECTOPHYRNOIDES VIVIPARA (N. Africa)—*Oxysomatium macintoshii*. 31. Bufonid (Africa)—*Orneoascaris chrysanthemoides*, and *Oxysomatium dogieli*. 32. "Toad" (Africa)—*Amplicaeum gedoelsti*, *A. involutum*, and *Foleyella duboisi*. 33. "Toad" (Columbia)—"*Filaria*" *columbi*. 34. "Toads" (Europe)—*Acleurostrongylus abstrusus*.

Records have been obtained from two genera (24 spp., and 5 supspp.) of the Bufoninae—representing every continent except Australia. 31 genera (81 spp.) of Nematoda (a number of them larval forms) are reported. Of these, *Rhabdias* (10 spp.), *Oswaldocruzia* (10 spp.), *Oxysomatium* (9 spp.), and *Aplectana* (7 spp.) are the genera best represented. In addition, eggs or larvae of three species normally found in mammals have been reported.

67. *The Clinical Diagnosis of Schistosomiasis mansonii by Rectoscopic Biopsy.* F. HERNANDEZ MORALES and JOSÉ F. MALDONADO, University Hospital and School of Tropical Medicine, San Juan, Puerto Rico.

The original method devised by Ottolina and Atencio (Revista de la Policlínica Caracas, Venezuela, XII(73): 1-35, Nov.-Dec. 1943) consists in obtaining a fragment, the size of a large grain of rice, from any point at the anterior half of the rectum, but particularly from the right dorsoventral valvular fold (plica coccylgea et sacralis) which serves as a better point of reference



for the rectoscopist. This fragment is digested in 4 per cent potassium hydroxide for three hours at 60 to 80 degrees C, centrifuged and the sediment examined under the microscope. We have modified this technique by examining the sample pressed between two slides without any previous treatment. By this modification we have obtained the following decided advantages over the original method: The method of examination is simplified and made more accurate; eggs are not altered in their actual appearance so that it is possible to differentiate between live and dead eggs and thus, with considerable control of the situation, to tell active from extinguished infections. The rectoscopic biopsy is a marked improvement over other methods of clinical diagnosis of schistosomiasis *mansoni*, particularly in case of very light infections. This is supported by the data presented by Ottolina and Atencio and our studies in progress at present.

68. *Schizomycetes parasitic in Paramecium bursaria*. RALPH WICHTERMAN, Temple University.

In cultures of a race of zoöchlorellae-free *Paramecium bursaria*, fairly large numbers of individuals were discovered which were parasitized with *Schizomycetes*. These spherical microorganisms, which stain with the Feulgen reaction measure less than a micron and exist in globular aggregates from approximately 3 to 45 microns in diameter in the endoplasm of *paramecia*. Individual specimens contain from one to twelve of these masses. Frequently four or fewer large masses of parasites are seen in which the body of the protozoön is misshapen. In severe cases of parasitism, the host assumes an abnormal pyriform shape with the aggregate of parasites at the wide end of the body.

In heavily parasitized *paramecia* the micronucleus is always present and appears normal but is not found in its typical position next to the macronucleus. Instead, the micronucleus is located at either end of the ciliate. On the other hand, the macronucleus appears to be attacked. In many cases the bacterial aggregate appears pressed against the macronucleus forming a cup-shaped depression and resulting in its hypertrophy or structural alteration. In such specimens the macronucleus is considerably smaller than in typical animals and suggests that macronuclear material is utilized in the metabolism of these microorganisms. It is easy to understand that the larger masses of parasites interfere not only with cyclosis but other trophic functions of the cell as well.

Generally, parasitized *paramecia* do not conjugate with members of the opposite mating type but one case was found in which a specimen with a bacterial aggregate measuring 32 microns was joined in conjugation at the stage of pronuclear exchange. Also a fission stage was found in which the macronucleus and micronucleus had completed division but the body of the protozoön had failed to constrict or divide. There is shown in these cases, the persistence of nuclear division in spite of parasitism. (Aided by a grant from the Committee on Research and Publication, Temple University.)

69. *Chemotherapy of Filariasis (Litomosoides carinii) in the Cotton Rat by the Administration of Stibanose (= Solustibosan)*. JAMES T. CULBERTSON AND ELIZABETH PEARCE, Columbia University.

Ten cotton rats all naturally infected with the filarial worm *Litomosoides carinii* and all presenting microfilariae in their tail bloods were repeatedly injected intramuscularly with stibanose, a pentavalent antimony compound. Five animals in one group were given 133.4 mg of drug daily for two weeks. The number of circulating microfilariae in these animals gradually declined and finally all the rats were negative on tail blood examination: one rat was negative on the twenty-first day from the beginning of treatment, two others were negative by the thirty-fifth day, and the remaining two were negative by the fifty-fourth day. These animals were autopsied after the tail blood was negative and their pleural spaces were searched for adult filarial worms. Adult worms, which were found in all rats, were in every case dead and embedded in exudate.

The second group of five rats was treated with 60 mg of drug daily for only six successive days. These animals were autopsied on either the fourteenth or twenty-first day after treatment began. Although all the rats still presented microfilariae in the tail blood at autopsy, the adult worms removed from the pleural space were dead and embedded in exudate. The drug evidently manifested greater activity on adult parasites than on microfilariae.

The filariasis of cotton rats can be eradicated by repeated administration of stibanose. Because of the excellent tolerance man is known to have for this drug, the trial of stibanose for the treatment of human filariasis is strongly indicated. (This work was done under contract with the Office of Scientific Research and Development.)

## AMERICAN SOCIETY OF PARASITOLOGISTS

## COUNCIL

*Officers for 1945*

ASA C. CHANDLER, Rice Institute .....	<i>President</i>
DONALD L. AUGUSTINE, Harvard University .....	<i>Vice-President</i>
JAMES T. CULBERTSON, Columbia University .....	<i>Secretary</i>
ROBERT M. STABLER, University of Pennsylvania .....	<i>Treasurer</i>

*Council Member Ex Officio†*

HORACE W. STUNKARD, New York University .....	<i>Chairman, Editorial Committee</i>
---	--------------------------------------

*Council Members at Large*  
*(with date of expiration of term)*

1948	GILBERT F. OTTO, Johns Hopkins University
1948	G. ROBERT COATNEY, National Institute of Health.
1947	HAROLD W. BROWN, Columbia University.
1947	CLAY G. HUFF, University of Chicago.
1946	HAROLD W. MANTER, University of Nebraska.
1946	EMMETT W. PRICE, U. S. Bureau of Animal Industry.
1945	RAYMOND M. CABLE, Purdue University.
1945	WILLARD H. WRIGHT, U. S. Public Health Service.

*Representatives of the Society on the Council of the American*  
*Association for the Advancement of Science*

JAMES E. ACKERT	HARLEY J. VAN CLEAVE
-----------------	----------------------

*Representatives of the Society on the Council of the Union*  
*of American Biological Societies*

AUREL O. FOSTER	ARTHUR C. WALTON
-----------------	------------------

*Editorial Committee of the JOURNAL OF PARASITOLOGY*

HORACE W. STUNKARD, <i>Chairman</i> .....	to serve until 1948
WILLIAM A. RILEY .....	to serve until 1948
DAVID H. WENRICH .....	to serve until 1948

*Editorial Board of the JOURNAL OF PARASITOLOGY*

1948	LLOYD A. SPINDLER, U. S. Bureau of Animal Industry.
1948	CHARLES W. REES, National Institute of Health.
1948	WILLIAM L. JELLISON, U. S. Public Health Service.
1947	LOWELL T. COGGESHALL, University of Michigan.
1947	JOHN T. LUCKER, U. S. Bureau of Animal Industry.
1947	NORMAN R. STOLL, Rockefeller Institute for Medical Research.
1946	BENJAMIN G. CHITWOOD, U. S. Bureau of Plant Industry.
1946	RUDOLF W. GLASER, Rockefeller Institute for Medical Research.
1946	PINCUS P. LEVINE, Cornell University.
1945	WILLIAM W. CORT, Johns Hopkins University.
1945	HAROLD KIRBY, University of California.
1945	BENJAMIN SCHWARTZ, U. S. Bureau of Animal Industry.

## LIST OF FORMER OFFICERS

*President*

1925	HENRY B. WARD*
1926	CHARLES W. STILES*
1927	RICHARD P. STRONG
1928	CHARLES A. KOFOID
1929	NATHAN A. COBB*

*Vice-President*

SAMUEL T. DARLING*
CHARLES A. KOFOID
EDWIN LINTON*
ROBERT HEGNER*
GEORGE R. LARUE

\* Deceased.

† Beginning in 1942, the Chairman, Editorial Committee, became ex officio member of Council.

1930	WILLIAM W. CORT	ERNEST CARROLL FAUST
1931	WILLIAM A. RILEY	ASA C. CHANDLER
1932	MAURICE C. HALL*	WILLIAM H. TALIAFERRO
1933	WILLIAM H. TALIAFERRO	FRED C. BISHOP
1934	ERNEST E. TYZZER	JAMES C. ACKERT
1935	CHARLES F. CRAIG	HARLEY J. VAN CLEAVE
1936	ROBERT HEGNER*	WILLIAM B. HERMS
1937	GEORGE R. LARUE	DAVID H. WENRICH
1938	FRED C. BISHOP	ELERY R. BECKER
1939	HORACE W. STUNKARD	HENRY E. MELENEY
1940	DAVID H. WENRICH	GOTTHOLD STEINER
1941	JAMES E. ACKERT	JUSTIN ANDREWS
1942	HENRY E. MELENEY	RUDOLF W. GLASER
1943	HENRY E. MELENEY	RUDOLF W. GLASER
1944	HENRY E. EWING	BENJAMIN SCHWARTZ

*Secretary-Treasurer*

WILLIAM W. CORT	1925; 1926; 1927; 1928; 1929
NORMAN R. STOLL	1930; 1931; 1932

*Secretary*

HORACE W. STUNKARD	1933-34; 1935-36; 1937
OLIVER R. MCCOY	1938-39; 1940-41; 1942
JAMES T. CULBERTSON	1942-43; 1944-

*Treasurer*

JUSTIN ANDREWS	1933-34; 1935-36
GILBERT F. OTTO	1937-38; 1939-40; 1944
L. E. ROZEBOOM	1941-42; 1942-44
ROBERT M. STABLER	1945-

*Council Members at Large*

PAUL BARTSCH	1925-28	JOHN F. KESSELL	1932-35
FRED C. BISHOP	1925-28; 1929-30	D. H. WENRICH	1932-35; 1936
ROBERT HEGNER*	1925-27	H. E. MELENEY	1933-36
CHARLES A. KOFOID	1925	NORMAN R. STOLL	1933-36; 1937-40; 1941
B. H. RANSOM*	1925	ELOISE B. CRAM	1934-37
WILLIAM A. RILEY	1925-26; 1928-30	WILBUR A. SAWYER	1934-37
CHARLES W. STILES*	1925; 1929-32	JAMES E. ACKERT	1935-38
ERNEST E. TYZZER	1925-26	EARL C. O'ROKE	1936-39
MAURICE C. HALL*	1926-29	JUSTIN ANDREWS	1937-40
WILSON G. SMILLIE	1926-27	HARLEY J. VAN CLEAVE	1938-41
HENRY B. WARD*	1926-29	ELERY R. BECKER	1939-43
FRANKLIN D. BARKER*	1927-30	EMMET W. PRICE	1939-43; 1944-
J. H. ST. JOHN*	1927-28	CLAY G. HUFF	1940-43; 1944-
W. H. TALIAFERRO	1928-31	HORACE W. STUNKARD	1940-43
ASA C. CHANDLER	1929-30; 1936-39	DONALD L. AUGUSTINE	1941-44
W. B. HERMS	1930-33	RAYMOND M. CABLE	1942-
BENJAMIN SCHWARTZ	1930-33	GILBERT F. OTTO	1942-44; 1945-
L. R. CLEVELAND	1931	WILLARD H. WRIGHT	1942-
W. W. CORT	1931-34; 1935-38	HAROLD W. BROWN	1944-
H. E. EWING	1931-32	HAROLD W. MANTER	1944-
ERNEST C. FAUST	1931-34; 1938-41	G. ROBERT COATNEY	1945-

*Editorial Committee of the JOURNAL OF PARASITOLOGY*

WILLIAM W. CORT, <i>Chairman</i>	1932-37	NORMAN R. STOLL, <i>Chairman</i>	1938-43
ROBERT HEGNER*	1932-34	WILLIAM A. RILEY	1934-37; 1938-43; 1944-
FRANCIS M. ROOT*	1932-34	WILLIAM H. TALIAFERRO	1934-37; 1938-43
		HORACE W. STUNKARD, <i>Chairman</i>	1944-
		DAVID H. WENRICH	1944-

\* Deceased.

*Editorial Board of the JOURNAL OF PARASITOLOGY*

CHARLES F. CRAIG	1932-33; 1934-37	OLIVER R. MCCOY	1936-39
MAURICE C. HALL*	1932-33	HENRY E. EWING	1937-40
HENRY B. WARD*	1932-33	JOHN F. KESSEL	1937-40
ASA C. CHANDLER	1932-34; 1935-38; 1939-42	HARLEY J. VAN CLEAVE	1937-40
CHARLES A. KOFOID	1932-34; 1935-38	WILLIAM W. CORT	1938-41; 1942
WILLIAM A. RILEY	1932-34	CORNELIUS B. PHILIP	1939-42
W. H. TALIAFERRO	1932-34	ERNEST E. TYZZER	1939-42
JAMES E. ACKERT	1932-35	HAROLD W. BROWN	1940-43
RICHARD P. STRONG	1932-35; 1936-39	HAROLD W. MANTER	1940-43
FRED C. BISHOP	1932-36	REGINALD D. MANWELL	1940-43
GEORGE R. LARUE	1932-36	RICHARD P. HALL	1941-44
DAVID H. WENRICH	1932-36; 1938-41	E. HAROLD HINMAN	1941-44
ERNEST C. FAUST	1933-37	JUSTUS F. MUELLER	1941-44
BENJAMIN SCHWARTZ	1933-37; 1938-41; 1942-	HAROLD KIRBY	1942-
ELERY R. BECKER	1934-35; 1936-39	BENJAMIN G. CHITWOOD	1943-
ROBERT MATHESON	1935-38	PINCUS P. LEVINE	1943-
		WILLIAM L. JELLISON	1945-
		CHARLES W. REES	1945-
		LLOYD A. SPINDLER	1945-

*List of Meeting Places*

1925	Kansas City	1932	Atlantic City	1939	Columbus
1926	Philadelphia	1933	Boston	1940	Philadelphia
1927	Nashville	1934	Pittsburgh	1941	Dallas
1928	New York	1935	St. Louis	1942	(New York, cancelled)
1929	Des Moines	1936	Atlantic City	1943	(No meeting)
1930	Cleveland	1937	Indianapolis	1944	Cleveland
1931	New Orleans	1938	Richmond		



IN MEMORIAM

CANDIDA M. AFRICA

CHARLES R. HAMILTON

HENRY B. WARD

## AMERICAN SOCIETY OF PARASITOLOGISTS

## LIST OF MEMBERS ELECTED

SINCE AUGUST 1, 1944<sup>1</sup>

- ALVES MEIRA, JOAO. Department of Tropical Medicine, Faculty of Medicine, University of São Paulo, Brazil.
- BAKER, ROLLIN H. Box 171, Eagle Lake, Texas.
- BALAMUTH, WILLIAM. Department of Zoology, Northwestern University, Evanston, Illinois.
- BLACKBURN, CLYDE CARLTON. Porter Sanitarium, Denver, Colorado.
- BRICEÑO-IRAGORRY, LEOPOLDO. Department of Bacteriology and Parasitology, Faculty of Medicine, Caracas, Venezuela.
- BUTTS, DONALD C. A. Department of Bacteriology, Georgetown University, Washington, D. C.
- CHAUHAN, BIRENDRA SINGH. Zoological Survey of India, Kaiser Castle, Benares, India.
- CARSON, HAMPTON L. Department of Zoology, Washington University, St. Louis, Missouri.
- CARTER, FRANKLIN. Ninth Medical Service Detachment (Lab.) A.P.O. 629, c/o P.M., New York, N. Y.
- CASIS-SACRE, GUILLERMO. Professor of Parasitology, School of Medicine, National University of Mexico, D. F.
- CHAPMAN, JEFF WATSON. Walterboro, South Carolina.
- CHEN, SUI-FONG. 733 North Broadway, Baltimore, Maryland.
- CHERNOFF, HARRY A. 55th Station Hospital, A.P.O. 782, c/o P.M., New York, N. Y.
- COFFIN, DAVID L. Department of Pathology, University of Pennsylvania, Philadelphia, Pennsylvania.
- CONLIN, JOHN FRANCIS. 103rd Evacuation Hospital, A.P.O. 403, c/o P.M., New York, N. Y.
- DEANE, LEONIDAS M. Department of Parasitology, Instituto Evandro Chagas, Belem, Pará, Brazil.
- DEANE, MARIA PAUMGARTEN. Department of Parasitology, Instituto Evandro Chagas, Belem, Pará, Brazil.
- DINGEE, RUTH FOSTER. Parasitology Laboratory, Medical College of South Carolina, Charleston, South Carolina.
- DRUDGE, J. HAROLD. Station Veterinarian, Camp Gordon Johnston, Florida.
- DUFF, FRATIS L. School of Aviation Medicine, Randolph Field, Texas.
- FERNANDEZ, FAUSTO. National Antimalarial Service, Health Department, Lima, Peru.
- FINKELSTEIN, SAMUEL MANUEL. 294 Washington St., Boston, Massachusetts.
- FORMAN, DOUGLAS N. Christian Medical Council for Overseas Work, 156 Fifth Avenue, New York, N. Y.
- FREITAS, MOACYR G. Department of Parasitology, Veterinary School of Minas Gerais, Belo Horizonte, Brazil.
- GIFFEN, HORACE K. Youngstown Hospital, Youngstown, Ohio.
- GORDON, WILLIAM H. 1102 David-Whitney Building, Detroit, Michigan.
- GREENE, DAVID G. 23rd General Hospital, A.P.O. 377, c/o P.M., New York, N. Y.
- GEISS, GEORGE W. 1160th Army Air Force, Base Unit, A.P.O. 603, Unit 1, c/o P.M., Miami, Florida.
- GERMAN, WILLIAM MCKEE. Department of Pathology, University of Cincinnati, Cincinnati, Ohio.
- GROCOTT, ROBERT G. Board of Health Laboratory, Ancon, Canal Zone.
- HOOD, MARION. Department of Public Health, Louisiana State University, New Orleans, Louisiana.
- JEFFERY, GEOFFREY M. Health and Safety Department, Tennessee Valley Authority, Wilson Dam, Alabama.
- JEWELL, ROSS L. Department of Pathology, Kansas State College, Manhattan, Kansas.
- KAUFMAN, BERNARD, JR. 2442 Broadway, San Francisco, California.
- KEEGAN, HUGH L. 7th Service Command Medical Laboratory, Ft. Omaha 11, Nebraska.
- KNIGHT, ALVA A. 122 S. Michigan Avenue, Chicago 3, Illinois.
- LEIGHTON, LESLIE H. 1802 Massachusetts Ave., Cambridge, Massachusetts.
- LIEBERTHAL, MILTON M. Moore General Hospital, Swannanoa, North Carolina.
- LUCAS, THOMAS L. Headquarters, 78th Infantry Division, A.P.O. 78, c/o P.M., New York, N. Y.
- LYONS, RANDOLPH. 3439 Prytania Avenue, New Orleans 15, Louisiana.

<sup>1</sup> To December 1, 1945. The last complete membership list was printed in *Journal of Parasitology*, 1942, 28 Suppl.: 35-50. The names of members elected thereafter up to August 1st, 1944, appeared in *Journal of Parasitology*, 30 Suppl.: 23 and 24, 1944.

- MARHOLIN, HERMAN E. 40th Malaria Survey Unit, A.P.O. 322, c/o P.M., San Francisco, California.
- MARTIN, DONALD S. Duke Hospital, Durham, North Carolina.
- MATERNY, MARGARET S. 2814 Pasadena St., San Diego 9, California.
- MAXWELL, ELMER STEPHENS. 190 N. Upper St., Lexington 15, Kentucky.
- MAXWELL, NANCY. Lankenau Hospital Research Institute, Philadelphia, Pennsylvania.
- MERRILL, GEORGE G. Department of Medicine, Johns Hopkins University, Baltimore, Maryland.
- NEHAUL, BALBIR BALL GREENE. Central Medical Laboratory, Georgetown, British Guiana.
- NEUMAYER, ELEANOR M. Zoological Division, U. S. Bureau of Animal Industry, Beltsville, Maryland.
- NEWTON, WALTER L. Health and Safety Division, Tennessee Valley Authority, Wilson Dam, Alabama.
- OHL, HARRY E. 15th Medical General Laboratory, A.P.O. 534, c/o P.M., New York, N. Y.
- PAPINEAU, ALBAN. Plymouth Clinic, Plymouth, North Carolina.
- PIMENTEL IMBERT, MANUEL FELIPE. International Hospital, Trujillo City, Dominican Republic.
- POINDEXTER, HILDRUS A. Department of Bacteriology, Howard University, Washington, D. C.
- PORTER, RICHARD J. School of Public Health, University of Michigan, Ann Arbor, Michigan.
- POWELL, CHARLES A. Massachusetts Memorial Hospitals, Boston, Massachusetts.
- PRATT, IVAN. Health and Safety Division, Tennessee Valley Authority, Wilson Dam, Alabama.
- QUINN, LOYD Y. Clinical Laboratory, Station Hospital, Boca Raton Air Field, Florida.
- REESE, JOHN D. Ninth Service Command Laboratory, Fort Lewis, Washington.
- ROBERTS, IRWIN H. P. O. Box 704, Albuquerque, New Mexico.
- RUSSELL, CATHERINE M. Department of Bacteriology, New York Medical College, New York 29, N. Y.
- RUSSELL, PAUL F. Army Medical School, Washington, D. C.
- SCHLOSSER, RALPH J. 420th Medical Composite Unit, A.P.O. 709, c/o P.M., San Francisco, California.
- SHRAPNEL, BLISS C. Gorgas Hospital, Ancon, Canal Zone.
- SIMPSON, WILLIAM F. Department of Biology, Catholic University of America, Washington, D. C.
- SMITH, MILTON S. 85th Station Hospital, A.P.O. 923, c/o P.M., San Francisco, California.
- SPIES, TOM DOUGLAS. Department of Medicine, University of Cincinnati, Cincinnati, Ohio.
- STORCH, SIDNEY. 96th Evacuation Hospital, A.P.O. 230, c/o P.M., New York, N. Y.
- TALMADGE, WALTER R. 117 S. Main St., Mahanoy City, Pennsylvania.
- TOMLINSON, WRAY J. A.P.O. 834, c/o P.M., New Orleans, Louisiana.
- VOGE, MARIETTA. Department of Zoology, University of California, Berkeley, California.
- WAGNER, EDWARD D. Biology Department, Atlantic Union College, South Lancaster, Massachusetts.
- WILLIAMS, THEODORE S. Department of Pathology and Parasitology, Tuskegee Institute, Alabama.
- WRIGHT, BERTRAND A. Armour Laboratories, Chicago 9, Illinois.
- WYBORNEY, EUGENE H. 36th Field Hospital, A.P.O. 245, c/o P.M., San Francisco, California.
- YOLLES, STANLEY F. 392 Medical Composite Unit, A.P.O. 869, c/o P.M., Miami, Florida.
- YOLLES, TAMARATH KNIGIN. 392 Medical Composite Unit, A.P.O. 869, c/o P.M., Miami, Florida.
- ZUCKER, ISADORE. Bruns General Hospital, Santa Fe, New Mexico.

APPLICATION FOR HOUSING ACCOMMODATIONS FOR ANNUAL  
MEETING, ST. LOUIS, MO., MARCH 28TH-30TH, 1946

All reservations for hotel rooms or other housing accommodations for the St. Louis Meeting must be filed not later than March 17th, 1946, with:

Housing Bureau, A.A.A.S.,  
910 Syndicate Trust Bldg.,  
St. Louis 1, Mo.

The name of each person must be listed for whom reservation is requested together with the type of accommodation desired. The definite dates and hours of arrival and departure must be submitted for each guest. The Jefferson Hotel will be the official headquarters of the American Society of Parasitologists and the meetings will be held at this hotel. Because of the shortage of rooms, it is urged that whenever possible two or more members submit applications for a single room, the rate at the Jefferson Hotel for one person is \$3.50 to \$5.00 per day. The names of other leading hotels and their rates for one person follow:

American	\$2.00 - \$3.00
Claridge	3.00 - 4.00
Lennox	3.00 - 5.00
Statler	3.50 - 5.00

The above information is from the Office of Harold A. Meyerhof, Executive Secretary, in charge of arrangements for the A.A.A.S. Meetings in St. Louis.

J. T. CULBERTSON, *Secretary*.



# The Journal of Parasitology

Volume 31

DECEMBER, 1945

Number 6

## SULFAMERAZINE THERAPY IN EXPERIMENTAL CECAL COCCIDIOSIS OF CHICKENS

MARION M. FARR AND EVERETT E. WEHR

Zoological Division, Bureau of Animal Industry,  
Beltsville Research Center, Beltsville, Md.

Levine (1939, 1940, 1941) tested sulfanilamide, sulfapyridine, and sulfaguanidine for the control of *Eimeria* infections in chickens and found that each of these sulfonamides had considerable coccidiostatic effect on several species of coccidia, but only sulfaguanidine had a marked inhibitory effect on infections with *E. tenella* and *E. necatrix*. He found, moreover, that the feeding of sulfaguanidine had no curative effect on chickens already infected with either of the two species named. Farr and Allen (1942) also tested sulfaguanidine against *E. tenella* infections in chickens and found that it had effective prophylactic properties, but that it was of little value as a treatment when administered after the appearance of blood in the dropping. Horton-Smith and Taylor (1942, 1943), working with sulfadiazine and sulfamethazine, reported favorable results from the use of these drugs against cecal coccidiosis when treatment was delayed as late as 72 hours and 96 hours, respectively, after experimental inoculation. Sulfadiazine was administered in the mash and sulfamethazine was given either in one-half gram daily doses, or in saturated aqueous solution as a substitute for the drinking water. Hawkins (1943, 1944) reported that sulfamethazine showed considerable promise as a therapeutic agent for cecal coccidiosis. Swales (1944), reported that sulfamerazine had a definite curative effect on *E. tenella* infections even after intestinal hemorrhage had begun.

The present paper contains data on the use of sulfamerazine both as a prophylactic and curative agent for cecal coccidiosis of chickens.

### EXPERIMENTAL PROCEDURE

The Rhode Island Red chickens used in these tests were obtained as day-old chicks from the poultry section of the Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Center, Beltsville, Md. They were immediately placed in electrically heated batteries and extreme care was taken to prevent them from acquiring an extraneous infection prior to experimental use. When removed from the batteries for use in an experiment, the birds were transferred to sterile all-metal brooders or cages. The brooders and cages were equipped with wire floors and with feeders and waterers that were attached to the outside to avoid contamination of the feed and water by the droppings. Newspapers were spread on the metal trays beneath the wire floors to facilitate the removal of the droppings.

Received for publication, July 14, 1945.

and were changed daily. The infective oöcysts of *Eimeria tenella* used to inoculate the birds were prepared as follows: Scrapings containing large numbers of oöcysts from the cecal walls of experimentally infected birds were placed in a 2.5 per cent solution of potassium dichromate where the oöcysts sporulated. The oöcysts were then washed in distilled water, and stored in a refrigerator for use when needed. The birds were inoculated by introducing the sporulated oöcysts into the crop by means of a graduated pipette.

Each of the tests was set up and carried out at different times under essentially identical conditions. Where there are exceptions, these are mentioned at the proper places in the text. The birds were weighed and distributed in groups of equal number and of approximately equal weight. Those groups of birds which were to receive oöcysts were inoculated on the same day so that the infection in each of the groups would be as nearly comparable as possible. Each of the treated groups of birds received medicated mash for seven days and then was placed on regular mash for an additional seven days. The number and designations of all groups of birds in each experiment and the day on which treatment was started, with the single exception of the uninoculated-treated group, are given in Table 1. This latter group in experiment 1 was started on the medicated mash on the second day of the test, while in experiments 2 and 3 the treatment period began on the first day of the test. All the birds were weighed every other day except Sundays. The surviving birds were killed and autopsied at the end of the second week of each test.

Wehr and Farr (1945) found, in their work with sulfaguanidine, that the two groups on treatment four and five days after inoculation suffered no recurrence of the disease during the week following cessation of treatment. In the present studies, therefore, these two groups were not held a full seven days after withdrawal of the medicated mash.

In the text which follows, the designation for each group shown in the table will be used.

#### RESULTS AND DISCUSSION

With respect to the time of appearance and amounts of blood passed by the inoculated groups of birds, the effect on the course of infection of *E. tenella* in birds treated with sulfamerazine is similar to that of those treated with sulfaguanidine (1945).

In test 1, the 1-T (treated one day before inoculation), T-0 (treated on the day of inoculation), T-1 (treated one day after inoculation) and T-2 (treated two days after inoculation) groups of birds passed no blood during the time the drug was being administered while the T-3 (treated three days after inoculation), T-5 (treated five days after inoculation) and IC (inoculated control) groups passed considerable blood during the first week after inoculation. However, during the next week, blood was present in the droppings of the T-0 and T-1 groups on the fifth day, of the 1-T, T-0 and T-1 groups on the sixth day and of the 1-T group on the seventh day after withdrawal of the medicated mash. One chicken of the T-1 group died on the fifth day after cessation of treatment. One of its wings was gangrenous, and there was a trace of blood in the cecal contents. This was the only bird involved in the four experiments that died from a cause other than coccidiosis. The mean weight gain of the UTC (uninoculated-treated control) group for the period covering the experiment was considerably less than that of the UUC

TABLE 1.—Effect of sulfamerazine on the course of infection with *E. tenella*. Each treated group received the drug for seven days

Test No.	Age of birds at start of test	Concentration of drug in feed	Groups	Number birds per group	Number oocysts administered	Mortality		Mean weight gains of survivors	Hemorrhage		No. of surviving birds with gross lesions of <i>E. tenella</i>		
						No. deaths	Per cent		1st week	2nd week	None	Slight	Marked
1	45	Per cent 1 1 1 1 1 1 None 1 None	1-T Treated 1 day before inoc.	7	138,000	0	..	Grams 167 176 200 157 171 60 139 114 269	0	++	..	..	7
			T-0 " on day of "			0	..		0	++	..	1	1
			T-1 " 1 day after "			1*	14		0	+	2	4	3
			T-2 " 2 days "			0	..		0	0	1	4	1
			T-3 " 3 days "			0	..		++	0	..	4	2
			T-5 " 5 days "			1	14		+++	0	..	1	2
2	14	1/4 1/4 1/4 1/4 1/4 None 1/4 None	IC Inoculated controls	25	52,500	0	..	90 110 109 110 103 97 77 93 177 131	+	+	3	7	18
			UUC Uninoculated treated controls			0	..		+	+	3	11	11
			" " " "			0	..		+	+	1	5	17
			T-1 " 1 day after "			0	..		+	0	1	7	17
			T-2 " 2 days "			1	4		+	0	2	4	15
			T-3 " 3 days "			5	20		++	0	2	4	14
3	14	1/4 1/4 1/4 1/4 1/4 None 1/4 None	T-4 " 4 days "	23	52,600	0	..	120 116 113 99 97 100 87 104 119 130	+	+	1	9	13
			T-5 " 5 days "			0	..		+	+	6	8	9
			IC Inoculated controls			0	..		+	0	6	7	10
			UUC Uninoculated treated controls			2	8		+++	0	5	4	8
			" " " "			4	17		+++	0	6	3	3
			T-4 " 4 days "			8	34		+++	0	10	3	11
4	26	1 1 None None	IC Inoculated controls	10	102,000	0	..	136 108 128 116 175	+	+	..	2	7
			UUC Uninoculated treated controls			0	..		0	+++	..	1	9
			" " " "			0	..		0	+	1	4	5
			T-1 " 1 day after "			6	60		+++	0	10	..	4
			IC Inoculated controls			0	..		0	+	..	..	..
			UUC Uninoculated untreated controls			0	..		0	0	10	..	..

\* This bird died of a wing infection.

† This bird died of cecal coccidiosis on the 5th day after removal of the medicated mash.

‡ Number of + indicates degree.

(uninoculated-untreated control) group for the same period. At autopsy, necrotic areas were present in the spleen and livers of half the treated birds. That these necrotic areas were probably due to the toxic effects of the drug is evidenced by their absence in the spleen and livers of any of the untreated birds. The drug, in spite of indications of toxicity, afforded the birds some protection against the disease, as only 1 of the 42 treated-inoculated birds died of coccidiosis, whereas 4 of the 7 inoculated controls died of this disease.

In tests 2 and 3, a mash containing only one-fourth of the amount of sulfamerazine fed in test 1 was used as the medicament. In both of these tests all of the inoculated groups of birds passed blood while on treatment. In test 2, blood appeared first in the droppings of the T-2, T-3, T-4, T-5 and the IC groups on the fifth day after inoculation; blood was not observed in the droppings of 1-T, T-0 and T-1 groups until one day later. The amounts of blood passed were relatively small in the groups placed on treatment on or before the second day after inoculation. Blood continued to appear in the droppings of the 1-T, T-5 and IC groups on the seventh day after inoculation, and in the 1-T and IC groups on the eighth day after inoculation. No blood appeared in the droppings of the T-0, T-1 and T-4 (treated four days after inoculation) groups on the seventh day after inoculation but reappeared on the eighth day. The reappearance of blood in the droppings of T-0, T-1 and T-4 groups after a lapse of one full day is probably not a recurrence due to the resumption of development of the coccidia, but merely a temporary cessation due to obstruction of the cecal openings or to some other cause. On the other hand, in experiment 3, traces of blood appeared in the droppings of the 1-T and T-0 groups on the fifth day after the removal of the medicated mash and in the droppings of the T-1 group on the sixth day. At autopsy, 10 of the treated birds of tests 2 and 3 of the treated birds of test 3 had necrotic areas in the spleen; only one treated bird of each of these two tests had liver lesions.

In test 2 there seemed to be a closer correlation between the time treatment was begun and the number of deaths than in test 3. This correlation was in the form of a progressive decrease in the percentage of deaths as the time between inoculation and treatment decreased. No deaths occurred in the birds of test 2 which were placed on treatment either one day before inoculation, at the time of inoculation, or one or two days after inoculation. Of the remaining birds, 96 per cent of those treated 3 days and 80 per cent of those treated 4 days after inoculation survived; 64 per cent of those treated 5 days and 40 per cent of the inoculated controls, respectively, survived. In test 3, there were no deaths among the birds placed on treatment either 1 day before, at time of, or 1 day after inoculation. Ninety-two per cent of the birds that were subjected to treatment 2 days after inoculation, 79 per cent of those treated 3 days, 83 per cent of those treated 4 days, and 66 per cent of those treated 5 days after inoculation survived; 66 per cent of the inoculated controls survived. It appears, therefore, that no great claims can be made for one-fourth per cent concentration of sulfamerazine as a control measure, when administered to birds later than the second day after inoculation. That the drug is apparently toxic even when fed in a concentration as low as one-fourth per cent is indicated by the lower mean weight gains of all the treated groups as compared with those of the UUC groups, and by the presence of spleen and liver lesions in some birds of both tests 2 and 3. The relatively greater number of deaths among the treated groups



and the smaller number in the inoculated controls of test 3 seem to indicate that these birds derived less benefit from the treatment than those of test 2.

The delayed appearance of blood in the droppings of the birds of groups 1-T, T-0 and T-1 of experiment 1, raised the question as to whether the birds had accidentally become reinfected or whether the drug had exerted a coccidiostatic effect. Test 4 was set up to throw some light on this point. Except for the number of groups of birds included, this experiment was performed in a manner similar to experiment 1. In this test each group of birds was transferred to a sterilized cage on the day the medicated mash was removed and three of the UUC birds were placed in the cage with the infected birds. The inoculated controls passed copious amounts of blood on the fifth and sixth days after inoculation and smaller amounts on the seventh day, while the treated birds passed no blood during this period. Six of the 10 inoculated controls died of cecal coccidiosis. The members of the 1-T group passed considerable blood on the fourth and fifth days after the removal of the medicated mash and were obviously sick at this time, one bird dying of cecal coccidiosis on the fifth day. The birds of the T-0 and T-1 groups also passed blood on the fourth and fifth days after cessation of treatment but were not nearly so sick as those of the 1-T group. At necropsy, all of the treated birds, except one, showed macroscopic lesions of cecal coccidiosis. The UUC birds appeared to be in good condition throughout the test and when examined post mortem, the ceca of these birds appeared normal and no coccidia were found in smears of the cecal contents. As a further check, the cecal contents of five of these birds were examined by the sugar solution flotation method. No oöcysts were found in four of the birds; a few oöcysts, less than 10, were recovered from the cecal contents of the fifth bird.

Since the UUC birds showed no clinical symptoms and no macroscopic lesions of cecal coccidiosis, it is clear that there was little possibility that reinfection occurred.

In case reinfection did take place, as may be indicated by the few oöcysts found in one of the UUC birds, it could not have produced the conditions observed in the treated birds.

The results of this experiment strongly indicate that the drug prevented the development of coccidia. That this may be the correct interpretation is shown by a study of the group which was placed on treatment one day before inoculation and, therefore, probably received the greatest amount of protection from the drug. The characteristic symptoms of cecal coccidiosis did not appear in this group until four days after the cessation of treatment and the disease was severe enough to cause the death of one bird on the fifth day.

With respect to behavior of the disease in chickens as influenced by the feeding of sulfamerazine mash, the results of experiments 1 and 3 substantiate those of experiment 4, except that during the second week the blood appeared one or two days later in the droppings of birds of experiments 1 and 3. In experiment 2, the presence of blood in the droppings of the birds during the second week did not seem to bear any relationship to the time of withdrawal of the medicated mash.

In-so-far as it is possible to ascertain from a clinical study of the living infected bird, the effect of sulfamerazine treatment on the course of infection with *E. tenella* is apparently similar to that of sulfaguanidine. However, the present studies show that the feeding of sulfamerazine was associated with much lower mean weight gains of the UTC groups as compared with those of the UUC groups, and the

presence in several of the birds of liver and spleen lesions. Wehr and Farr (1945) did not encounter these ill effects in chickens fed sulfaguanidine.

From a consideration of the life cycle of *E. tenella*, a species requiring 7 days to complete its development within the avian host, it seems that the two drugs in question exert a static effect on the development of the coccidial parasites.

#### SUMMARY AND CONCLUSIONS

1. Experiments were conducted with 593 chickens to determine the effect of sulfamerazine on the course of infection with *Eimeria tenella*.

2. Sulfamerazine administered in the mash in a concentration of one per cent materially reduced mortality from cecal coccidiosis. This percentage of the drug was toxic as indicated by the presence of spleen and liver lesions and of retarded weight gains.

3. One-fourth per cent sulfamerazine mash was an effective prophylactic agent for cecal coccidiosis and also was of benefit as a treatment when fed within two days after inoculation. This percentage was considerably less toxic than one per cent but an occasional bird showed spleen and liver lesions; some loss in weight gains was noted.

4. When fed before or within one day after inoculation one per cent sulfamerazine was markedly coccidiostatic as long as it was being administered. Within four to six days after cessation of this treatment clinical coccidiosis developed.

#### REFERENCES

- FARR, M. M. AND ALLEN, R. W. 1942 Sulfaguanidine feeding as a control measure for cecal coccidiosis of chickens. J. Am. Vet. Med. Assn. 100: 47-51.
- HAWKINS, P. A. 1943 Sulfamethazine treatment of cecal coccidiosis. Poultry Sc. 22: 459.
- 1944 Sulfamethazine in the treatment of *Eimeria tenella* infections in poultry. J. Parasitol. 30 Suppl.: 6.
- HORTON-SMITH, C. AND TAYLOR, E. L. 1942 Sulphamethazine and sulphadiazine treatment in cecal coccidiosis of chickens. A preliminary note. Vet. Rec. 54: 516.
- AND ——— 1943 Saturated solution of sulphamethazine as a substitute for drinking water in the treatment of cecal coccidiosis in chickens—a preliminary note. Vet. Rec. 55: 109-110.
- LEVINE, P. P. 1939 The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell Vet. 29: 309-320.
- 1940 The effect of sulfapyridine on experimental avian coccidiosis. J. Parasitol. 26: 233-235.
- 1941 The coccidiostatic effect of sulfaguanidine (sulfanilyl guanidine). Cornell Vet. 31: 107-112.
- SWALES, W. E. 1944 On the chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens. Canad. J. Res., D, 22: 131-140.
- WEHR, E. E. AND FARR, M. M. 1945 Effect of sulfaguanidine on the course of infection of chickens for *Eimeria tenella*. J. Parasitol. 31: 359-365.

## EFFECT OF SULFAGUANIDINE ON THE COURSE OF INFECTION IN CHICKENS WITH *EIMERIA TENELLA*

EVERETT E. WEHR AND MARION M. FARR

Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture,  
Beltsville Research Center, Beltsville, Md.

Of the sulfonamides which have been tested experimentally against avian coccidiosis, sulfaguanidine has shown considerable promise as a prophylactic in this disease. By a series of tests in which this drug was administered in the mash, Levine (1941) found that sulfaguanidine prevented infection of birds with certain of the species of coccidia which complete their development in the small intestine and markedly reduced the severity of infection with *Eimeria tenella* and *E. necatrix*, which complete their development in the ceca. The institution of sulfaguanidine treatment three days after experimental inoculation did not, according to Levine, alter the course of infection with *E. tenella* and *E. necatrix*. Farr and Allen (1942) reported that birds which had received sulfaguanidine in mash, in either 1 or 2 per cent concentration, for three days before and for nine days after inoculation with *E. tenella* showed no symptoms or severe lesions. They reported, moreover, that birds to which either 3 or 5 per cent sulfaguanidine in the mash was given for one week beginning at the time of the appearance of blood in the droppings were not significantly benefited, although oöcyst production was sharply reduced.

The purpose of the studies reported in this paper was to ascertain the effect, on the course of infection with *Eimeria tenella*, of sulfaguanidine in mash when administered for 7 consecutive days, beginning one day prior to, on the day of, and one, two, three, four and five days, respectively, after inoculation.

### MATERIALS AND METHODS

The birds used in these tests were obtained as day-old chicks from the poultry section of the Animal Husbandry Division, Beltsville Research Center, Beltsville, Md. When 16 days old, the chicks were weighed, banded, and then sorted on the basis of their weights, into the required number of groups, the lightest and heaviest birds being discarded so as to make for greater uniformity among the groups. Each group was composed of an equal number of birds, the total weights of the birds of each group being very close. A wire-bottomed brooder unit, which was equipped with feed and water containers attached to the outside of the unit, served as a confinement unit for each group of birds. Newspapers were spread over the metal trays beneath the wire floors to catch the droppings and to facilitate their removal each day. Two days were allowed for the birds to become accustomed to their new surroundings and to permit the return to normal feeding before the experiment was begun. It was possible sometimes within this period to detect birds which, for one reason or another, might detract from the uniformity of the group as a whole and thereby influence the results. These birds were eliminated, therefore, from the experiment. The total number of birds for the different groups of each test as listed in Table 1 is different because of the final check-up and elimination.

Received for publication, July 14, 1945.

TABLE 1.—Effect on the course of infection with *Eimeria tenella* of sulfaguanidine. Each treated group received the drug for 7 consecutive days

Exp. No.	Age of birds at start of exp.	Conc. of drug in mash	Groups	Number birds per group	Number oöcysts given per bird	Haemorrhage		Mean wt. gains of survivors	Mortality		Surviving birds having—		
						1st week	2nd week		No.	Per cent	No lesions	Slight lesions	Marked lesions
1	16	0.5	1-T, Treatment began 1 day before inoculation	20†	68,000	++	+	Grams	1	5	No.	No.	No.
			T-1, " " 2 days " after	19	"	+	+	110.8	4	21	5	5	11
			T-2, " " 3 " " "	19†	"	+++	..	99.4	6	31	2	2	11
			T-3, " " 4 " " "	20†	"	+++	..	93.0	12	60	1	1	9
			T-4, " " 5 " " "	20	"	+++	..	95.8	17	85	0	0	6
			T-5, " " 5 " " "	20	"	+++	..	59.0	17	85	1	1	3
			IC, Inoculated Controls	20	"	+++	..	69.0	18	90	1	1	1
			UTC, Uninoculated-Treated Controls	18	0	0	..	80.5	0	..	0	0	0
			UUC, Uninoculated-Untreated Controls	18	0	0	..	129.7	0	..	18	0	0
								133.9					
2	16	1.2	1-T, Treatment began 1 day before inoculation	20	101,000	+	++	155.0	0	..	12	6	2
			T-0, " " on same day as inoculation	20†	"	+	+	154.3	0	..	12	7	0
			T-1, " " 1 day after inoculation	21	"	+	+	133.9	0	..	2	18	0
			T-2, " " 2 days " "	22	"	++	..	136.6	1	4.5	2	9	1
			T-3, " " 3 " " "	22	"	+++	..	121.2	3	13.6	1	4	11
			T-4, " " 4 " " "	21	"	+++	..	106.8	10	47.6	2	4	13
			T-5, " " 5 " " "	22	"	+++	..	103.3	18	81.8	1	1	10
			IC, Inoculated Controls	22	"	+++	..	87.5	20	90.9	1	0	2
			UTC, Uninoculated-Treated Controls	22	0	0	..	169.0	0	..	22	0	0
			UUC, Uninoculated-Untreated Controls	22	0	0	..	158.9	0	..	22	0	0

\* Plus sign indicates relative amount of blood passed.

† Not all of surviving birds examined.



The care of the birds was assigned to one person who kept feed and clean water before them all the time and removed the droppings daily.

Rhode Island Red chicks infected with *E. tenella* were used as a source of oöcysts. The birds were inoculated with just enough sporulated oöcysts to produce at the most only traces of blood. Such lightly infected birds usually showed very little disturbance from the infection and yielded large numbers of oöcysts. All donor birds were sacrificed on the seventh or eighth day after inoculation and the cecal pouches removed. The scrapings of the ceca containing the unsporulated oöcysts were immediately placed in a 2.5 per cent potassium dichromate solution and allowed to remain at room temperatures until the oöcysts sporulated. The oöcysts were then washed repeatedly in water. The test birds were infected by introducing the oöcysts into the crops with a graduated pipette.

For measuring the oöcyst output, the dilution method employed by Farr and Allen (1942) was used. Oöcysts were recorded as oöcysts in thousands per gram of feces. While the method used for detecting the presence of oöcysts in the droppings lacked the refinement and accuracy of other methods, it had the advantages of simplicity and speed that are required when numerous samples have to be examined over a short period.

Each experiment was conducted independently and at different times. The setup of experiment 2 differed from that of experiment 1 in that (1) a higher percentage of the drug was fed in the mash; (2) a greater number of oöcysts was administered to each bird; and (3) an extra group, treated at the time of inoculation, was added.

Sulfaguanidine in mash was fed continuously for seven days. Nonmedicated mash was substituted for the medicated mash for the remaining period of the experiment which, in the case of experiment 1, was 6 days and, in the case of experiment 2, 7 days. The purpose of holding the treated birds for one week after the withdrawal of the medicated mash was to ascertain if any interference in the course of the infection might result from the medication.

Observations on the development of possible toxic effects of the drug, the time of appearance of blood in the droppings of each of the inoculated groups, and the general behavior of the birds, were noted daily. Dead birds were removed daily from the brooder units and promptly examined for signs of toxicity and for presence of coccidia. The cause of death, if ascertained, was recorded.

For convenience, symbols, as explained in Table 1, are used to designate particular groups within an experiment.

#### RESULTS AND DISCUSSION

The results of two experiments involving the administration of sulfaguanidine in mash in two different concentrations to chickens before and after experimental inoculation with *Eimeria tenella* are presented in summarized form in Table 1. Table 2 shows the oöcyst output in thousands per gram of feces of the IC (inoculated controls), 1-T (treated 1 day before inoculation), T-0 (treated on same day as inoculated), T-1 (treated 1 day after inoculation), and T-2 (treated 2 days after inoculation) groups of experiment 2 over a period of successive days after the withdrawal of the medicated mash. Irrespective of the differences in the structural setup of the two experiments, the results are, except in degree, similar.

The data as presented permit certain broad conclusions: (1) that the greatest benefit from sulfaquanidine treatment was derived by the 1-T, T-0, T-1, T-2, and T-3 (treated 3 days after inoculation) groups; (2) that the drug was apparently responsible for the reduced amounts of blood passed by the 1-T, T-0 and T-1 groups during the period of treatment; and (3) that the drug apparently exerted a coccidiostatic effect was demonstrated by the reappearance of blood in the droppings of the 1-T, T-0, and T-1 groups, as well as by the gradual increase in oöcyst output, during the week after the withdrawal of the medicated mash; (4) that the reappearance of blood in the droppings of the 1-T, T-0, and T-1 groups during the week following the withdrawal of the medicated mash was probably not due to reinfection of the birds. This has been satisfactorily demonstrated, it is believed, in our paper on sulfamerazine therapy (1945).

The lower mortality percentages and the greater weight gains of the 1-T, T-0, T-1, T-2 and T-3 groups justify the first conclusion. Little or no benefit from sulfaquanidine therapy was derived by either the birds of experiment 1 which were placed

TABLE 2.—Oöcyst output of the IC, 1-T, T-0, T-1 and T-2 groups of experiment 2 in thousands of oöcysts per gram of droppings

Number of days after inoculation	IC	1-T	T-0	T-1	T-2
7	1,695	0	...	...	..
8	2,520	0	0	...	..
9	256	50	0	...	..
10	115	95	3	0	..
11	83	186	70	23	0
12	66	803	63	76	0
13	63	420	440	90	3
14	70	406	550	442	10
15	52	396	353	316	63
16	30	336	310	153	73
17	3	...	153	126	23
18	10	...	...	50	6

on medicated mash later than the third day or by the groups of birds of experiment 2 which were started on treatment later than the fourth day after infection, as the percentage of mortality in each of these groups was much higher than that of any of the other groups. The absence of any deaths in the groups 1-T, T-0 and T-1 and comparatively few deaths in the groups T-2, T-3, and T-4 (treated 4 days after inoculation) of experiment 2 testify to the greater severity of the infection in corresponding groups of experiment 1.

In an effort to find a cause for the greater severity of the infection in the treated birds of experiment 1, the principal points to be considered are the numbers of oöcysts administered and the concentration of the drug fed to the birds of each experiment. It is apparent that the number of oöcysts administered had no bearing on the intensity of the infection in the birds of the two experiments because (1) a larger number of oöcysts were administered to the birds of experiment 2, and (2) the percentage of mortality in the IC group of birds of each experiment was almost identical. This leaves, therefore, for consideration only the concentration of the drug.

The fact that the lower concentration of the drug in the mash fed to the birds of experiment 1 apparently afforded less protection than the higher concentration given to the birds of experiment 2 is based on the following observations: (1) the 1-T, T-0 and T-1 groups of experiment 2 survived the infection *in toto*, whereas the 1-T and T-1 groups of experiment 1 (the T-0 group was not represented) had

1 and 4 deaths, respectively; (2) the T-2, T-3, and T-4 groups of experiment 2 sustained fewer losses than these same groups of experiment 1; (3) there was a larger percentage of surviving birds with no gross lesions of *Eimeria tenella* in experiment 2.

Part of the data presented in Table 1 is plotted on semi-logarithmic paper and shown as Fig. 1. These data show that when treatment was instituted on or before one day after experimental inoculation, there were no deaths as in experiment 2 or

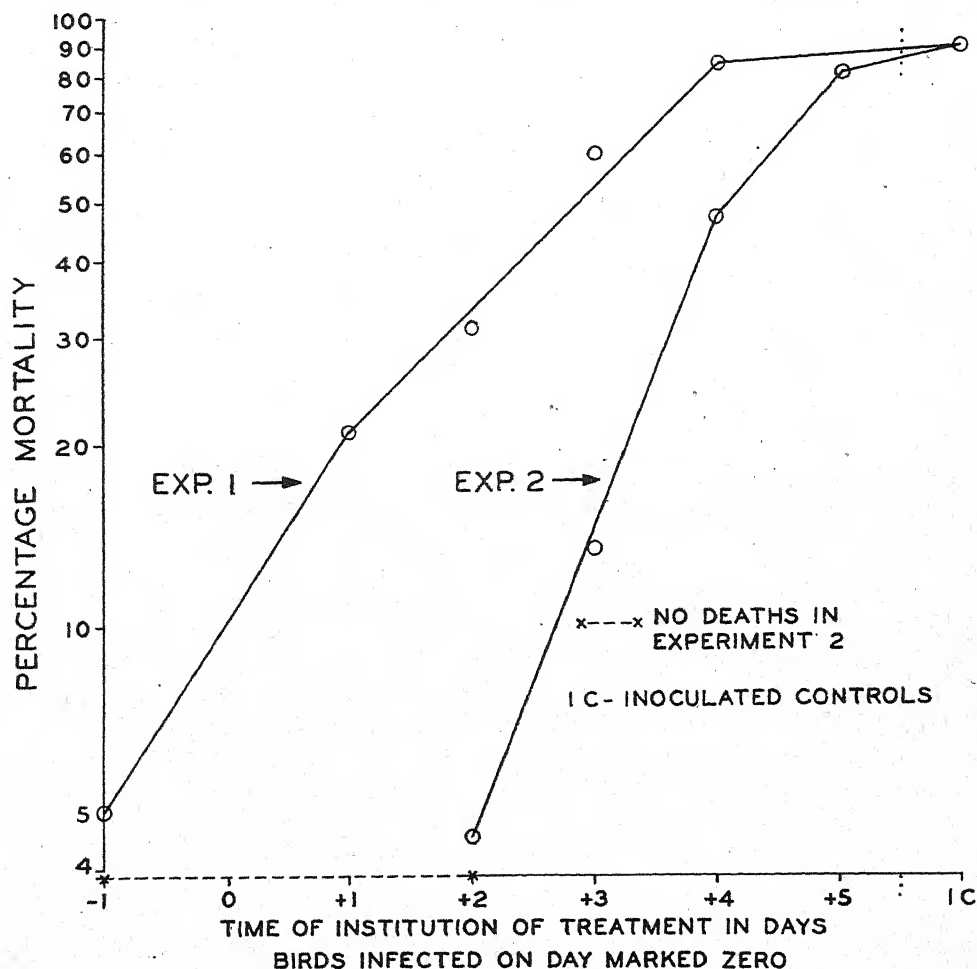


FIG. 1. Graph showing relationship between increase in mortality rate and time of treatment.

relatively few as in experiment 1. However, when treatment was instituted later than one day after experimental inoculation, the death rate increased with delay in drug treatment. In those groups in which the institution of treatment was delayed until the fourth day as in experiment 1 and the fifth day as in experiment 2 after inoculation, the increase in the death rates did not differ materially from those of the inoculated controls. Closer scrutiny of the chart shows that the death rate increased most rapidly during the period from plus 1 to plus 4 days in experiment 1

and from plus 2 to plus 4 in experiment 2. Furthermore, since the increase in death rate is represented on the logarithmic chart as a linear relationship to delay in inception of treatment, this would indicate, because of the nature of this type of chart, that each day in delay of institution of treatment is associated with a regular increase in death rate within these periods.

The amounts of blood passed by the birds of the two experiments were similar and served as evidence that the treatment, at least in certain of the groups, in some way interfered with the normal course of the infection. On the fifth day after experimental infection, blood was present in the droppings of all the inoculated groups of experiment 1, in small amounts in the 1-T and T-1 groups, and in considerably greater amounts in the other groups. Blood continued to be passed in small amounts in the droppings of the birds of all groups through the seventh day. The 1-T and the T-1 groups were the only birds to pass blood in the droppings during the second week of infection. Small amounts of blood were observed in the droppings of the 1-T group on the sixth day and of the T-1 on the fourth day after the withdrawal of the medicated mash. On the fourth day after inoculation, small amounts of blood were seen in the droppings of the IC, T-3, T-4, and T-5 (treated 5 days after inoculation) groups of experiment 2. However, on the fifth day, traces of blood appeared in the droppings of the 1-T, T-0, T-1 and T-2 groups, while larger amounts appeared in the droppings of the IC, T-3, T-4 and T-5 groups. Blood in small amounts was passed with the droppings of 1-T, T-0 and T-1 groups on the sixth day after inoculation and in larger amounts in all the other groups; none was observed in any of the groups on the seventh day. Traces of blood were observed in the droppings of only the 1-T, T-0 and T-1 groups on the fourth and fifth days after the discontinuance of feeding of medicated mash, of the T-0 group on the sixth day, and of the 1-T group on the seventh and eighth days.

The delayed appearance of blood and the passage of smaller amounts of blood in the droppings of the 1-T, T-0 and T-1 groups of experiment 2, and the reappearance of blood in the droppings of the above groups during the week following the withdrawal of the medicated mash are unquestionably associated with treatment and are indicative of a static action of the drug against coccidia. A similar phenomenon was observed by Farr and Wehr (1945) as a result of feeding sulfamerazine to chickens infected with *Eimeria tenella*. The gradual increase in oöcyst output of the 1-T, T-0, T-1 and T-2 groups and a steady decline in the oöcyst output of the IC groups following withdrawal of the medicated mash demonstrate further the possible static action of the drug.

Oöcyst counts on those groups which were placed on medicated mash later than the second day after experimental inoculation are not included in Table 2 because those birds did not appear to be benefited to the same degree as those placed on sulfaguanidine mash earlier. A study of the table will show that the peak of oöcyst production in the IC group was reached on the eighth day after experimental inoculation, with a steady decline, thereafter, except for two days when there was a slight rise, until the discontinuance of oöcyst counts on the eighteenth day after infection. In contrast to this, the treated groups showed no oöcysts for the first day or two after the removal of the medicated mash, but there was a rather rapid increase in the oöcyst output by these groups for the next few days, the peak being reached on the sixth and seventh days after the withdrawal of the medicated mash; then a steady



decline took place. It should be noted that as the period between inoculation and treatment increased there was a progressive decrease in the total number of oöcysts produced, with the lowest production in the group placed on medicated mash two days after inoculation.

The results of the present studies, in so far as they relate to the effect of the drug on the oöcyst output of the treated birds are in close agreement with those reported by Levine (1939, 1940). This investigator demonstrated a suppression of the oöcyst output in birds infected with *Eimeria praecox*, *E. maxima*, *E. mitis*, or *E. hagani* as the result of the feeding of 0.3-gm dose of sulfanilamide daily. However, within one or two days after the cessation of treatment with this drug, the number of oöcysts in the droppings began to increase until the peak was reached on the third or fourth day. Levine found also that while chickens infected with *E. praecox*, *E. mitis*, or *E. hagani* were being treated with 0.7 per cent sulfapyridine mash, the oöcyst output was relatively small. But when the medicated mash was removed "a gradual increase in the oöcyst discharge occurred and peaks were reached five days later." In none of his experiments on the use of sulfaguanidine did Levine include groups of birds to which treatment was administered on all of the days included in the present studies. Therefore, no comparison of the results of Levine's work with sulfaguanidine and those presented herein can be made.

#### SUMMARY AND CONCLUSIONS

1. Experiments were conducted with 388 birds to determine the effect of sulfaguanidine on the course of infection with *E. tenella*.
2. The feeding of 0.5 per cent sulfaguanidine mash for 7 consecutive days decidedly benefited young birds when treatment was begun 1 day before or within 2 days after experimental infection.
3. The feeding of mash containing 1.2 per cent sulfaguanidine for 7 consecutive days prevented deaths among groups of birds placed on treatment one day prior to, on the day of, and one day after inoculation. The administration of this mash as late as the third day after infection markedly reduced mortality.
4. The results of the present studies apparently demonstrate a coccidiostatic action of sulfaguanidine.

#### REFERENCES

- FARR, M. M. AND ALLEN, R. W. 1942 Sulfaguanidine feeding as a control measure for cecal coccidiosis of chickens. J. Am. Vet. Med. Assn. 100: 47-51.
- AND WEHR, E. E. 1945 Sulfamerazine therapy in experimental cecal coccidiosis of chickens. J. Parasitol. 31: 353-358.
- LEVINE, P. P. 1939 The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell Vet. 29: 309-320.
- 1940 The effect of sulfapyridine on experimental avian coccidiosis. J. Parasitol. 26: 233-235.
- 1941 The coccidiostatic effect of sulfaguanidine (sulfanilyl guanidine). Cornell Vet. 31: 107-112.

## VARIATIONS IN RESPONSE TO FILARIFORM LARVAE OF *ANCYLOSTOMA CANINUM* IN THE SKIN OF MAN

GEORGE W. HUNTER, III,<sup>1</sup> AND C. BROOKE WORTH<sup>2</sup>

Army Medical School, Washington, D. C.

### INTRODUCTION

Reports on the reaction of the skin of man to penetration by filariform larvae of *Ancylostoma caninum* (Ercolani, 1859) Hall, 1913, have been in conflict on several points, particularly with regard to the subsequent behavior and fate of the worms. White and Dove (1929) described a transient papular dermatitis, clearly distinct from the clinical appearance of burrows that follow penetration by larvae of *Ancylostoma braziliense* de Fairo, 1910. Heydon (1929) and Fülleborn (1930), however, reported a more prolonged reaction, apparently produced by deep penetration and an ensuing aimless migration or "creeping eruption" of the parasites through the superficial tissues. These reports raised the following questions: (1) are there separate strains of *A. caninum*, possessing variable abilities to invade the skin of man? or (2) does the human subject vary constitutionally in his ability to arrest the invasion of a parasite which may be considered uniform within its species?

The following work, carried out with a single culture of *A. caninum* larvae, presents evidence which would answer the latter question in the affirmative.

### MATERIALS AND METHODS

Two volunteers, one of whom had a past medical history of multiple allergies, infected themselves on the volar surface of their right forearms, each with 1000 to 1500 *A. caninum* filariform larvae obtained from Dr. W. W. Cort, Johns Hopkins University. These larvae were secured from feces of laboratory-housed dogs known to harbor a pure culture of this species of worm. The larvae were washed until macroscopically clean, but no bacteriostatic agents were used.

The resultant infections were unexpectedly violent in character. Surgical consultation elicited the opinion that a bacterial cellulitis was superimposed on the helminthic process, microorganisms having penetrated the cutaneous sites of entry along with the larvae. Heydon (1929) remarked of one of his cases that, if he had been unaware of the true etiology, he would naturally have suspected a septic process.

The symptoms and progress of each subject are presented and compared below.

### RESULTS

#### 1. *Subject A.* No past allergic history.

A prickling sensation was noted during the time of application of the larvae. Within forty-five minutes the area was distinctly erythematous and itching had developed. The inflammation extended rapidly, accompanied by increasing pruritus, and in forty-eight hours virtually the entire anterior aspect of the forearm from wrist to elbow was involved. Urticarial lesions, some sinuous in outline, were present

Received for publication, May 3, 1945.

<sup>1</sup> Major, Sanitary Corps, A.U.S.

<sup>2</sup> Captain, Medical Corps, A.U.S.

radially, while at the site of penetration numerous small papules were evident. The forearm felt tense and axillary lymph nodes were slightly tender.

On the third day the forearm was indurated and tender. Sulfadiazine, 4 grams daily, was instituted. During the next three days pain, induration and erythema began slowly to subside. Sulfadiazine dosage was then cut to 1 gram twice daily for the next four days.

By the twelfth day the forearm appeared and felt normal except for a few small areas where slight tension still existed. The subject had remained ambulatory throughout and had had no systemic reaction except for a slight elevation of temperature (less than one degree F.) on the second and third days. His subsequent reactions consisted of occasional transient pruritus and edema (urticarial wheals) at or

TABLE 1.—Total and differential white blood cell counts

Subject A—Non-allergic							
Day	WBC	Polys	Lymphs	Monos	Basos	% Eos	Eos/cmm
3	9,900	76	17	5	0	2	198
7	7,800	71	23	4	1	1	78
10	7,100	67	28	4	1	0	...
26	6,300	73	21	5	0	1	63
85	8,850	62	24	5	0	9	797
92	7,000	65	22	7	0	6	420
99	7,200	52	43	2	0	3	216
120	6,200	57	33	5	0	5	310

Subject B—Allergic							
Day	WBC	Polys	Lymphs	Monos	Basos	% Eos	Eos/cmm
8	8,700	71	24	1	0	4	348
10	12,400	75	18	0	0	7	868
12	8,900	68	20	8	0	4	356
29	12,800	62	12	0	0	26*	3,328
33	11,900	50	44	0	2	4	476
72	12,000	50	30	4	1	15	1,800
77	11,800	56	25	5	0	14	1,652
84	11,600	50	30	10	0	10	1,160
92	10,050	68	14	7	0	11	1,105
98	9,900	58	26	4	0	12	1,188
102	10,250	71	15	4	0	10	1,025
106	14,250	74	14	5	0	7	998
120	8,700	52	38	4	0	6	522
181	13,400	64	23	5	0	8	1,072
209	.....	78	12	4	0	6	....

\* Sulfa rash: onset of "flu."

near the site of inoculation. These episodes occurred at increasing intervals of time for several months, each one lasting a day or two. On the twenty-seventh day he had one such lesion excised in order to determine the presence or absence of a migrating larva as its cause. Serial sections of this tissue, fixed in Zenker's, showed no evidence of a parasite in the epidermis or corium, though some sections exhibited slight lymphoid and plasma cell infiltration around the papillae. The pathologist's diagnosis was "slight chronic inflammatory reaction."

Total and differential white blood cell counts made during the course of the infection are presented in Table 1 and plotted in Fig. 1. Other laboratory work consisted chiefly of repeated stool examination to search for *A. caninum* ova. Although there were no gastro-intestinal symptoms suggestive of the presence of adult worms, this species has been known rarely to reach the intestine of man and become mature (Manalang, 1925; Dove, 1932). Results of stool examinations are presented in Table 2.

2. *Subject B.* Past allergic history as follows. Allergic coryza (late summer

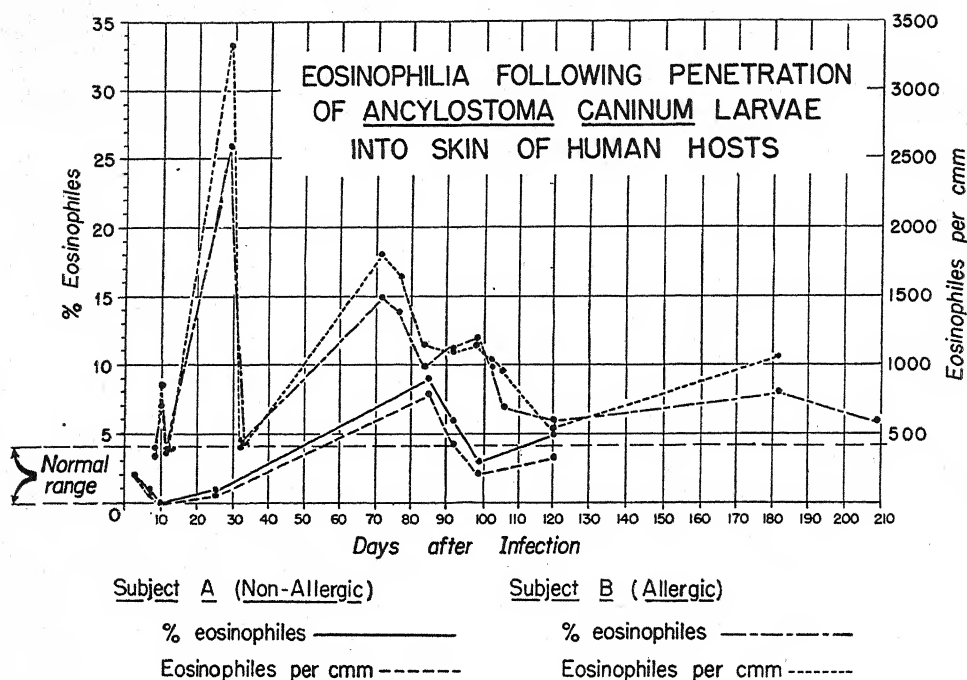


FIG. 1. Range of eosinophilia in allergic and non-allergic subjects following cutaneous penetration of infective *Ancylostoma caninum* larvae.

variety, eastern United States) from infancy, decreasing in early adult life. Chief allergen ragweed. No asthma. Food sensitivities: celery, parsley, egg, hazel-nut, carrot and many others to a less degree. Sensitive to horse and cat dander. Sensitive to atropine. Siblings and paternal lineage also allergic.

This subject had ascariasis twenty-three years previously. It cannot be said, however, whether or not he thus became sensitive to a nematode-type protein. The infection at that time was asymptomatic.

Following the application of *A. caninum* larvae he felt less immediate pruritus than the first subject, and the erythema was also milder. On the second day macules and papules were visible at the points of entry. This was followed on the third day by a spreading inflammation, slight itching and moderate superficial tenderness. The papules were becoming vesicular and the epitrochlear lymph node was palpable. The skin was tense, hot and dry. Sulfadiazine, 4 grams daily, was begun.

On the fourth day burrows were clearly evident. These appeared first as

TABLE 2.—Stool examinations

Day	Subject A	Subject B
3	Negative for helminths	.....
19	Negative for helminths	.....
30	<i>Endolimax nana</i>	.....
31	<i>Endolimax nana</i>	.....
37	.....	Negative
88	<i>Giardia lamblia</i>	.....
89	<i>Giardia lamblia</i>	.....
97	<i>G. lamblia</i> and <i>E. nana</i>	.....
104	.....	Negative
105	<i>Giardia lamblia</i>	.....
108	<i>Giardia lamblia</i>	.....



vaguely outlined, slightly elevated, tortuous, erythematous lines but subsequently were marked by a succession of vesicles which ruptured and became crusted, each in turn.

From the fourth to the sixth day sulfadiazine was continued and the swelling receded slightly. On the seventh day, however, the subject experienced a sudden onset of deep induration of the entire forearm with severe aching pain throughout the thickness of the member. On admission to surgical service of the hospital he had the following symptoms: pitting edema of forearm with small vesicles; minimal signs of bacterial infection; palpable epitrochlear and axillary nodes; no pain in forearm with extension of fingers and wrist; temperature, pulse and respiration normal. Impression: cellulitis of right forearm.

The subject was placed on a routine in which sulfadiazine was continued. In addition wet boric dressings, with a heating pad, were applied constantly. Daily soaking in a hot tapwater whirlpool bath for about an hour was also instituted. Shelmire (1928) has suggested that heat be contraindicated in the therapy of creeping eruption due to its tendency to increase the activity and wandering of the larvae.

The surgeon's further notes were:

"9th day. Edema slowly subsiding. X-ray of forearm negative.

"19th day. Edema seems to be subsiding; however, the parasites appear to be active, migrating toward the tip of the digits.

"24th day. Skin rash; appears due to the sulfadiazine; this will be discontinued. Temperature, pulse and respiration normal. Whirlpool continued.

"30th day. Severe case of flu, temperature 102° F. No findings in chest. Bed care.

"34th day. Temperature, pulse and respiration normal. General condition good. Right arm OK.

"38th day. Discharged, cured. Final diagnosis: cellulitis, acute, suppurative, right forearm, severe—secondary to dog hookworm."

Other laboratory findings on Subject B during the period of hospitalization included negative blood Kahn reaction on 14th day, bleeding and clotting times 1 and 4 minutes respectively on 8th day, blood sulfadiazine levels of 8.9 and 5.7 on 8th and 10th days, respectively, negative urines on 10th and 12th days, and red blood values averaging 4,500,000 RBC and 80–85 per cent hemoglobin throughout the period of the experiment.

For the next several months the subject had numerous recurrences of symptoms, although none of these required further medical care. At times there were generalized or localized swellings; at others there were renewed burrowings. These episodes were most noticeable following active use of the right forearm (chopping, sawing, etc.). On the 53rd day a long burrow appeared on the ulnar side of the wrist. On the 59th day the dorsum of the hand was swollen. On the 69th day there was again swelling of the dorsum of the hand, followed on succeeding days by swelling first of the wrist (volar aspect) and then of the elbow (lateral aspect). These episodes were attended by erythema with central urticarial wheals of irregular serpentine shape but without definite burrows. Further symptoms—with swellings, burrows or both—occurred on the 76th, 78th, 102nd, 177th and 208th days, usually as a sequel of direct trauma to the arm or of an upper respiratory infection.

## SEROLOGICAL STUDIES

Otto (1939) and Sarles (1938) have shown that precipitates form about filariform larvae immersed in hyperimmune serum of experimental animals, while serum from nonimmune hosts exhibits no such activity. Since the subjects of the present study had experienced massive infection, experiments were performed on both their sera on the 77th and 87th days. Living *A. caninum* filariform larvae were immersed in serum on glass slides under cover glasses sealed with vaseline. Controls were set up both in normal serum and in tap-water. The slides were incubated at 37.5° C and studied intermittently for two days. No immune precipitates formed about the larvae. The mortality rate of larvae in the experimental serum was not significantly different from that of the controls; all larvae were dead at the end of the study period, whereas Otto's controls lived for two weeks.

## DISCUSSION

Hookworm species adapted to man as a final host penetrate beneath the superficial layers of the skin rather rapidly, sometimes without noticeable host reaction, then enter the blood stream and eventually migrate to the intestinal lumen. The depth to which "foreign" species penetrate the skin of man is apparently subject to some variation, depending as it would seem, on two separate factors, namely, the degree of adaptation of the hookworm species to man and the degree of resistance offered by the individual human host. Thus it has been known for some time that *A. braziliense*, the commonest cause of creeping eruption, wanders far through the cutaneous layers and survives for periods up to three months or longer (Kirby-Smith, Dove and White, 1926). *Uncinaria stenocephala*, the European dog hookworm, is somewhat less adapted to man, since Fülleborn (1927), while reporting lesions similar to those produced by *A. braziliense*, states that their duration is only three or four weeks. *Ancylostoma caninum* would seem usually to occupy the lowest position in the scale of invasiveness: Ellenbogen (1930) was unable to secure penetration of his skin by normal means; after blistering with cantharidin a few larvae did enter, but none produced a burrow and only one caused a small inflamed swelling at the point of entry. Other writers have described the typical *A. caninum* lesion as a papular or very short linear one that disappears within fourteen days.

The questions of allergy and sensitization in the rôle of these reactions are not fully answered by any means. Fülleborn (1930), who experienced distant wandering of a single *A. caninum* larva that entered the fingernail bed, said that he was sensitized as a result of previous *Strongyloides stercoralis* infection as well as a series of polyvalent helminth antigen injections. This result might seem to parallel the findings in the case of Subject B in this report. However, Heydon's case (1929), in whom wandering occurred also, was unresponsive to intracutaneous injection of *Ancylostoma* extract in spite of the fact that he had harbored an intestinal infection by *A. duodenale* for the previous two years.

Stumberg (1932) failed to mention eosinophiles in tissue sections through burrows of *A. caninum*, describing only the infiltrative findings common to mechanical trauma. Sandground (1939), Sandler (1938) and Taliaferro and Sarles (1937), however, all noted eosinophiles among other white cell accumulations about larvae that were being attacked or destroyed by the host. Eosinophiles—both in the blood and in solid tissues—may function as phagocytes, but this activity is a more character-

istic property of neutrophilic leucocytes. Therefore the presence of infiltrating eosinophiles in tissues invaded by helminths need not be regarded as a significantly added barrier to the worms' progression. It is, more probably, a chemotactic response, since the chief function of eosinophiles is believed to be to desensitize the host to parenteral foreign proteins (Ringo, 1938), a concept entirely in accord with the association of eosinophilia with allergy. Allergic individuals, therefore, do not arrest the worms more effectively, or less so, than do non-allergic ones, and the explanation of the observed clinical variations must lie elsewhere.

A further note on the mechanism of host resistance to these worms may be made. *Ancylostoma braziliense* burrows are characteristically continuous with the points of entry of the larvae. *Ancylostoma caninum* burrows, as observed in Subject B and reported by Fülleborn and by Sandground, may be discontinuous, that is, the larvae penetrate to deeper tissues and later come to the surface at some distance from the points of entry (seven to eight inches distant in Subject B). It would seem therefore that *A. braziliense*, possessing invasive properties against a poorly resistant host, can wander rather freely through the skin despite the tissue's attempts to block it. *Ancylostoma caninum*, on the other hand, may be arrested at once, with resultant papular or negative reactions; or, if it can get through to deeper and less sensitive layers, it may then wander until it accidentally reaches the superficial skin once more. This would explain at once the two clinical types of reaction that have been described and the recurrent swellings experienced by Subject B over a period of many months.

#### SUMMARY

1. Two volunteers infected the skin of their right forearms with many filariform larvae of *Ancylostoma caninum*.

2. Subject A, without a past allergic history, experienced a short period of discomfort (under two weeks) with swelling and pruritus, followed by several months during which transient urticarial wheals appeared at or near the site of inoculation. He showed a maximum of 9% eosinophiles three months later.

3. Subject B, with a past history of multiple allergies, was hospitalized during most of the first month due to acute swelling of the entire forearm. A bacterial cellulitis was undoubtedly present, but in addition burrows appeared, extending discontinuously for seven or eight inches from the site of inoculation. During the succeeding several months he experienced occasional episodes of local or distant swelling, marked by burrow-like wheals. From a maximum of 26% he progressed into a gradually subsiding eosinophilia which fell below 10% after three and one-half months.

4. Neither subject showed eggs of *A. caninum* in the stools on the occasions these were examined; nor were there gastrointestinal symptoms suggestive of the presence of adult worms.

5. The host-reaction to various nematode larvae causing creeping eruption is discussed.

#### ACKNOWLEDGMENTS

The authors express their thanks to Captain Reinard Harkema, Sanitary Corps, A.U.S., and to Mrs. Virginia G. Warren for critical reading of this paper.

#### REFERENCES

- DOVE, W. E. 1932 Further studies on *Ancylostoma braziliense* and the etiology of creeping eruption. Am. J. Hyg. 15: 664-711.

- ELLENBOGEN, V. 1930 Experimenteller Beitrag zur Frage der durch die Larven von *Ancylostoma caninum* beim Menschen verursachten Hauterscheinungen. Klin. Wschr., Berlin 9: 1583-1585.
- FÜLLEBORN, F. 1927 Durch Hakenwurmlarven des Hundes (*Uncinaria stenocephala*) beim Menschen erzeugte creeping eruption. Arb. Tropenkrankh. (Festschr. B. Nocht) Hamburg 121-133, 6 pl.
- 1930 Über die durch die Larve von *Ancylostoma caninum* verursachten Hauterscheinungen. Giorn. Clin. med. (Festschrift für Prof. Gabbi).
- HEYDON, G. B. 1929 "Creeping eruption" or larva migrans in North Queensland and a note on the worm *Gnathostoma spinigerum* (Owen). Med. J. Australia 2: 583-591.
- KIBRY-SMITH, J. L., DOVE, W. E., AND WHITE, G. F. 1926 Creeping eruption. Arch. Derm. and Syphil. 13: 137-173.
- MANALANG, C. 1925 Hookworm campaign in Cebu. Philippine J. Sc. 27: 483-493.
- OTTO, G. F. 1939 A serum antibody in dogs actively immunized against the hookworm, *Ancylostoma caninum*. Am. J. Hyg. 31: 23-27.
- RINGOEN, A. R. 1938 Eosinophile leucocytes and eosinophilia, in *Handbook of Hematology*, Hal Downey, Editor, pp. 181-229. Paul B. Hoeber, Inc., New York.
- SANDGROUND, J. H. 1939 Creeping eruption in the Netherlands East Indies caused by the invasion of the larvae of *Ancylostoma braziliense*. Geneesk. Tschr. Ned. Indië 79: 805-810.
- SANDLER, I. L. 1938 Creeping eruption; report of two cases and brief review of the literature. Med. Ann. District of Columbia 7: 245-247.
- SARLES, M. P. 1938 The in vitro action of immune rat serum on the nematode *Nippostrongylus muris*. J. Infect. Dis. 62: 337-348.
- SHELMIRE, B. 1928 Experimental creeping eruption from a cat and dog hookworm (*A. braziliense*). J. Am. Med. Assn. 91: 938-944.
- STUMBERG, J. E. 1932 Cutaneous retention of infective larvae of the dog hookworm, *Ancylostoma caninum*, and the inflammatory reaction to skin penetration. Am. J. Hyg. 15: 186-205.
- TALLAFERRO, W. H. AND SARLES, M. P. 1937 Cellular reactions during immunity to *Nippostrongylus muris* in the rat. J. Parasitol. 23: 561 (Abst.).
- WHITE, G. F. AND DOVE, W. E. 1929 A dermatitis caused by larvae of *Ancylostoma caninum*. Arch. Derm. and Syphil. 20: 191-200.



# A NEW MITE, *LAELAPS APLDONTIAE*, FROM *APLONTIA*<sup>1</sup>

WILLIAM L. JELLISON

Sanitarian (R), United States Public Health Service

Several collections of mites from the mountain beaver, *Aplodontia rufa* (Rodentia: Aplodontidae), represent an undescribed species of the family *Laelaptidae*. This species is placed provisionally in the genus *Laelaps* although it does not have the characters of this genus in the restricted sense in which *Laelaps* is defined today. Particularly the ventral plate of the female differs from that of *Laelaps s.s.*, in having six pairs of spinelike setae instead of four and in its elongate shape.

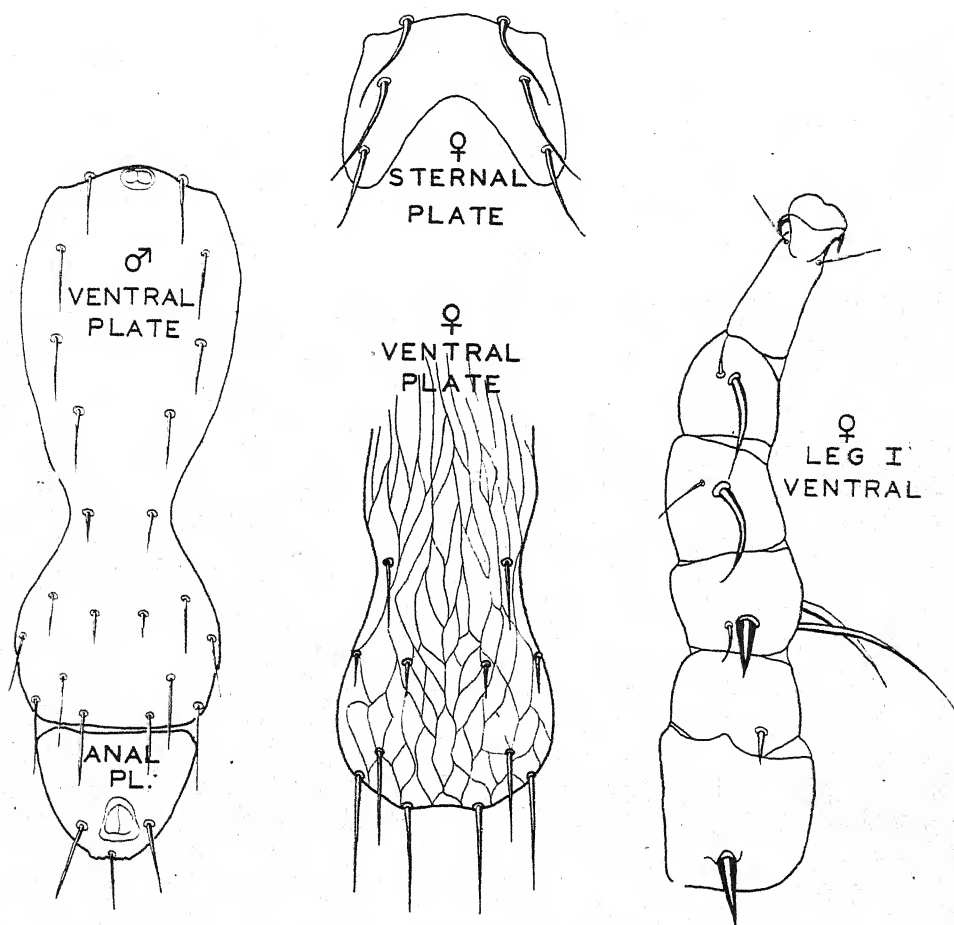


PLATE I. *Laelaps apodontiae*, n. sp. Ventral and anal plates of male. Sternal and ventral plates, and leg I of female.

*Laelaps apodontiae*, n. sp.

*Female*: Length 1 mm. The sternal plate, which is figured, is horseshoe-shaped with the anterior margin slightly convex and the posterior margin deeply concave. The sternal plate bears

Received for publication, May 2, 1945.

<sup>1</sup> From the Rocky Mountain Laboratory (Hamilton, Mont.), National Institute of Health.

3 pairs of setae of which the anterior pair is marginal. There is a fourth pair of ventral bristles posterior to the plate and opposite coxae III. The combined genito-ventral plate is figured. Its anterior margin is indistinct; it bears 4 rows of setae as follows, 2-4-2-4. The last row is marginal. The anal plate is separated by its width from the ventral plate. It is trapezoidal in shape and bears a pair of setae opposite the anal opening and a median posterior ventral seta. All legs are well armed with spines and bristles. Leg I is figured. It bears on the dorsal surface 2 long heavy bristles on article III and 4 spines on article IV. The tarsus has a pair of claws and a rounded pulvillus. There are ventral spines on all articles of leg I but are heaviest on articles I and III. The peritreme is long and slender, extending from the middle of coxa I to the posterior side of coxa IV. The spiracular opening is opposite the posterior margin of coxae III. Metapodal plates present but small. Chelicera of the pincer type with the immoveable finger slender and the moveable finger robust and slightly enlarged at the tip.

*Male:* Length 0.9 mm. Sternal, genital and ventral plate fused into one sclerite which is figured. Nine rows of setae on this sclerite arranged 2-2-2-2-2-4-2-2-4. The anterior row is marginal. The anal plate is contiguous with the ventral plate and is figured. Spines and bristles on legs are more robust than in the female. Article IV of leg I bears a specialized bristle which is broad at the base but bifurcates into a short, sharp prong and a long sinuous filament. Chelicerae flagelliform with long slender chitinous bases that extend posteriorly within the body to coxae IV.

The type series consists of the female holotype from *Aplodontia rufa*, Seattle, Washington, April 3, 1940, collected by W. A. Dalquest, and is from the collection of the United States National Museum. Allotype male, 2 paratype males, and 2 paratype females from *A. rufa*, Curry County, Oregon, May 26, 1935, from the collection of the Rocky Mountain Laboratory.

The type host, which is probably the normal host of this mite, is phylogenetically one of the most isolated of living rodents. It is the sole survivor of the superfamily APLODONTOIDEA, a family whose fossil history dates back to the upper Eocene (Wilson, 1937). Characteristic parasites of such an isolated mammal should be very distinctive. This is true of two monotypic genera of fleas, *Dolichopsyllus* Baker and *Trichopsylloides* Ewing, which are parasites of *Aplodontia*. It is also true of this new mite. No ANOPLURA or MALLOPHAGA have been reported from *Aplodontia*.

#### REFERENCE

- WILSON, R. W. 1937 Pliocene rodents of western North America. Carnegie Institution of Washington Pub. No. 487, pp. 21-73.

# THE CULTIVATION OF *TRICHOMONAS AUGUSTA* (PROTOZOA) FROM FROGS

D. H. WENRICH

Zoological Laboratory, University of Pennsylvania

## INTRODUCTION

According to the compilations of Walton (1941) and of Morgan (1944), *Trichomonas augusta* has been reported from 19 species in 7 genera of urodele amphibians, from 33 species in 9 genera of anurans, and from one species of lizard. *Trichomonas batrachorum* is also fairly common in amphibia, but has not been reported from so many kinds of hosts: 2 species in 2 genera of urodeles, 22 species in 7 genera of anurans, 2 lizards and one snake. From these lists one might assume that *T. augusta* is the more hardy species, and the more successful in adapting itself to different kinds of hosts. Yet, if one places a frog's rectal contents containing both species into some of the culture media commonly employed for trichomonad flagellates, *T. batrachorum* will grow in almost any of them while *T. augusta* usually does not grow at all or, if it does develop for a short time, it will soon be overwhelmed by the more rapidly growing *T. batrachorum*. This situation has been a challenging one and on several occasions I have assigned to graduate students the problem of trying to cultivate *T. augusta*. Until recently these efforts have not succeeded in maintaining this species in vitro for more than about 2 weeks. Unexpected success was attained in the winter of 1944-45.

## METHODS

Cultures were maintained in ordinary test tubes with the usual cotton plugs, fluid media with added nutrients being employed. The basic fluid was either one-half strength Ringer's solution or the modified Ringer of Drbohlav (1925). For the most part the nutrients were gastric mucin and Bacto Loeffler's dried blood serum.

Specimens of *Rana pipiens* were the host animals. Original inoculations were from the recta of the frogs and contained the bacteria present therein. The usual precautions were taken to exclude organisms from the air. All cultures were maintained at room temperature.

Survival of the flagellates in individual culture tubes was prolonged by the addition of nutrient materials as needed and by adding distilled water to replace that lost by evaporation. In original cultures the mucin was dissolved by boiling, customarily in a concentration of 0.2%. Added nutrients were usually in the dry condition, an amount equal to about 0.2% being added at any one time. However, a stock of 0.4% gastric mucin in distilled water was kept on hand to use when both a nutrient and replacement water were to be added. For examination, a drop from near the top of the fluid, as well as one from the bottom, with sediment, was removed with a sterile pipette. Usually the flagellates were concentrated at the bottom, but often they were in the upper levels and occasionally were more numerous there than at the bottom. The following culture symbols will be used in the table and in the text: R<sup>1</sup> refers to regular Ringer's fluid, R<sup>2</sup> to Drbohlav's modification; L is used for the Loeffler's dried blood serum; M for gastric mucin and W for water; BH stands for

Bacto dried beef heart, E-M for Bacto dried egg-meat medium, Li for Bacto liver powder, Ri for Bacto powdered rice, and Cer for cerophyl.

I am indebted to the Cerophyl Laboratories of Kansas City, Mo., for a sample of cerophyl and to the Wilson Laboratories, Chicago, Ill., for a supply of gastric mucin.

#### OBSERVATIONS

Having kept a number of strains of *Trichomonas batrachorum* under cultivation for some months, and desiring to obtain a new strain of this species, a frog was killed and opened, on Dec. 9, 1944. When the rectum was removed it was apparently empty. It was cut in two and a little juice was squeezed from one part into R<sup>2</sup> on a slide. One specimen of *T. augusta* and a few of *Hexamitus* were observed. Assuming that a few individuals of *T. batrachorum* might be lurking in the rectal lumen, a piece was dropped into R<sup>2</sup> containing 0.2% gastric mucin (R<sup>2</sup>M). Examination of the culture two days later revealed only *Hexamitus*, but 2 days after that a few individuals of *T. augusta* appeared. The numbers of *T. augusta* increased for several days and then began to decline. On Dec. 18, R<sup>2</sup> containing 0.1% of bile salts was added but the trichomonads did not increase. On Dec. 24 a piece of liver from another frog was dropped into the tube and in a few days the numbers had increased again. Then a decline set in. On Dec. 28, distilled water containing 0.4% of mucin was added and the numbers again increased. Water and mucin were added once more on Jan. 5, 1945, but on Jan. 9 the trichomonads appeared to be degenerating. Some dry beef heart was then added but *T. augusta* was not seen after that date. *Trimitus parvus*, which had appeared on the 5th day of culture persisted until the 66th day.

*Trichomonas augusta* had thus been shown to multiply in this culture which contained at first a piece of the rectum of the host, and later a piece of liver from another frog, and had persisted for 31 days. It seemed possible, therefore, that culture success might be attained if pieces of host tissue, especially pieces of rectum, were added to the media usually employed. However, a transplant into R<sup>2</sup>M to which a piece of frog liver was added kept this species alive for only 12 days. Transplants into R<sup>2</sup>M, R<sup>2</sup> with 0.03% peptone, R<sup>2</sup>BH with 0.05% tryptone, and R<sup>2</sup>M on Bacto-Entamoeba-medium slant did not keep *T. augusta* alive longer than 14 days, while *Trimitus* persisted for 184 days in the transplant containing originally a piece of frog liver.

This limited success in inducing *T. augusta* to multiply in a culture tube led to further attempts to cultivate it. But whenever *T. batrachorum* was present, it gradually increased in numbers till it apparently overwhelmed *T. augusta*, which did not persist longer than 24 days in any of the cultures containing both species. In the case of one frog, however, examined on Dec. 27, 1944, *T. batrachorum* did not appear in the cultures, and *T. augusta* was maintained as long as the experiment continued, until June 25, 1945.

Only 2 original cultures were made from this frog: A in R<sup>2</sup>M (modified Ringer's with 0.2% gastric mucin), and B in R<sup>2</sup>L, but the inoculum of each consisted of a piece of rectum with its contents. A number of years ago a student had found that *T. augusta* tolerated 0.4% NaCl better than stronger or weaker solutions. Consequently most of the subcultures were made in one-half strength Ringer's ( $\frac{1}{2}$ R<sup>1</sup>). At the start of this series of cultures, it was deemed desirable to add new nutrient mate-



rials frequently and to vary them somewhat, hence gastric mucin and Loeffler's serum were commonly added alternately every 2 to 4 days. After the trichomonads had apparently become adjusted to cultural handling, various other methods were tried. Certain of the 26 cultures set up are shown in the table.

As seen in the table, original culture B, started in R<sup>2</sup>L, was still positive when the cultures were abandoned, June 25, 1945, the flagellate having persisted in this tube for 180 days, while original culture A had become negative a few days previously. This is interesting, since the A culture had maintained a higher density of population through most of the period involved. The additions to A were limited to mucin, Loeffler's serum and water (see table) while B had also Bacto liver powder once and rice powder twice. Rice powder was first added when this culture was 72 days old and the next day many of the flagellates contained starch grains. Rice powder was

TABLE 1

Showing records of certain of the cultures into which *Trichomonas augusta* was inoculated. Original cultures are designated by a capital letter; subcultures by a number indicating the transplant generation followed by a small letter referring to each individual tube. Abbreviations: R<sup>2</sup>, modified Ringer's;  $\frac{1}{2}$ R<sup>1</sup>, one-half strength regular Ringer's; W, distilled water; L, Loeffler's dried blood serum; M, gastric mucin; BH, Bacto dried beef heart; E-M, Bacto dried egg-meat medium; Ri, Bacto powdered rice; Li, Bacto liver powder; Cer, Cerophyl.

Culture	Date	Original medium	Mucin added	Loeffler's added	Other nutrients	Water added	Days of survival
A	Dec. 27, '44	R <sup>2</sup> M	30	31	....	11	177
A/1a	Jan. 8, '45	$\frac{1}{2}$ R <sup>1</sup> M	26	32	....	8	168*
A/1b	" 8, '45	$\frac{1}{2}$ R <sup>1</sup> MBH	3	..	BH6	2	54
A/1f	Feb. 14, '45	WML	2	1	....	..	9
A/1f <sup>2</sup>	Mar. 3, '45	WML	12	12	....	3	77
A/1g	" 3, '45	WML	..	..	....	..	..
A/1g <sup>2</sup>	" 9, '45	WML	1	..	....	..	8
A/1g <sup>3</sup>	" 27, '45	WML	13	14	....	2	83
A/1i	" 3, '45	$\frac{1}{2}$ R <sup>1</sup> L	..	6	....	..	52
A/2a	Jan. 19, '45	$\frac{1}{2}$ R <sup>1</sup> MBH	18	14	BH6, Ri6	8	157*
A/2b	" 20, '45	$\frac{1}{2}$ R <sup>1</sup> M	25	25	Cer2	7	156*
A/3a	Feb. 1, '45	$\frac{1}{2}$ R <sup>1</sup> M	26	23	Ri4	8	144*
A/4a	Mar. 1, '45	$\frac{1}{2}$ R <sup>1</sup> M	23	..	....	1	72
A/4b	" 5, '45	$\frac{1}{2}$ R <sup>1</sup> L	..	4	....	..	12
B	Dec. 27, '44	R <sup>2</sup> L	28	26	Li1, Ri2	5	180*
B/1a	Jan. 26, '45	$\frac{1}{2}$ R <sup>1</sup> M	26	21	....	5	148
B/1b	Mar. 9, '45	$\frac{1}{2}$ R <sup>1</sup> MLRi	18	17	Ri3	3	168*
B/1c	" 9, '45	WE-M	18	2	Ri3, Cer2	3	101*
B/2b	Apr. 7, '45	$\frac{1}{2}$ R <sup>1</sup> MRi	18	..	Ri3	1	75
B/2c	" 7, '45	$\frac{1}{2}$ R <sup>1</sup> LRi	..	23	Ri5	1	77
B/3b	" 14, '45	$\frac{1}{2}$ R <sup>1</sup> LRi	..	21	Ri5	2	74*

\* Still positive when abandoned, June 25, 1945.

then tried on other cultures and it was found that *T. augusta* will, in culture, ingest relatively large amounts of rice starch, until most of the cytoplasm is filled with granules.

Since the alternate addition of mucin and dried serum proved to be capable of maintaining this species, a test of the capacity of each substance separately was made. Culture A/1i was started with  $\frac{1}{2}$ R<sup>1</sup>L and the dry serum added every few days. The numbers gradually declined but persisted for 52 days. Culture A/4b, treated the same way, remained positive only 12 days. Culture A/2c was started with  $\frac{1}{2}$ R<sup>1</sup>ML. Dry serum was added once when it was 8 days old, but all subsequent additions were of mucin. This culture remained positive for 104 days. Later these two nutritive substances were tried separately with starch. B/2b, with mucin and starch, remained positive for 75 days, M having been added 18 times and rice powder 3 times. B/2c, containing L and starch at the beginning, had L added 23 times and rice powder 5 times; it remained positive for 77 days. B/3b, a transplant of B/2c, started with

serum and rice powder, remained positive until abandoned on June 25, lasting 74 days. Rice powder was added 5 times and serum 21 times.

Bacto beef heart served fairly well as a nutritive material. Culture A/1b, started in  $\frac{1}{2}$ R<sup>1</sup>MBH, had mucin added 3 times and beef heart 6 times. The trichomonads persisted 54 days. Culture A/2a, a transplant from A/1b, was initiated in  $\frac{1}{2}$ R<sup>1</sup>MBH and it remained positive for 157 days, until discontinued on June 25. But in this case beef heart was added only 6 times, while mucin was added 18 times and rice powder 6 times.

Some attempts were made to grow *T. augusta* in cultures made up in water. Culture A/1f set up in WML remained positive for 9 days. Eight days later it was reinoculated and remained positive for 77 days. Another attempt in WML was made (A/1g) but the flagellates did not survive. After 6 days this tube was reinoculated (A/1g<sup>2</sup>), and the trichomonads survived for 8 days. Ten days later this same tube was again inoculated (A/1g<sup>3</sup>) and the flagellates persisted for 83 days. Increase in osmotically active ingredients was probably a factor in improving the "conditioned" medium in these reinoculated tubes. Water and Bacto whole egg did not prove effective, but water and Bacto egg-meat (B/1c) did better, the culture remaining positive for 101 days, until abandoned; but during this period mucin was added 18 times, dry serum twice, rice powder 3 times, and cerophyl twice. Addition of cerophyl was not followed by any marked increase in numbers but addition of rice powder usually was. It was found advisable to add the rice powder in very small amounts and at relatively long intervals, since larger amounts appeared to be detrimental.

A trial was made of the technique of Kofoed and Swezy (1915) and of Rosenberg (1936): namely, that of sealing with vaseline the rectal contents from a frog in Ringer's fluid under a cover glass on a slide. After some days *T. augusta* gradually disappeared while *T. batrachorum* increased in numbers and eventually became the only species present. The latter persisted for about 4 months and then died out.

#### DISCUSSION

Existing literature provides a number of reports of the cultivation of *Trichomonas augusta* but they may not be reliable. Often when the contents of a frog or other amphibian are examined, they will be swarming with this species. *T. batrachorum* may also be present but in numbers too small to be noted. Usually, when such material is placed in culture media and examined some days later, large numbers of *T. batrachorum* may be present, while *T. augusta* has disappeared. If the examination is not too critical, the impression will be gained that the latter species has been successfully cultivated. Several times during the past 20 years in my laboratory, students have tried to cultivate *T. augusta* but always with disappointing results. The somewhat accidental success that is reported above may not be easily repeatable, but at least this one strain has been successfully cultured. At present the indications are that the following conditions are likely to result in the satisfactory culture of this species: (1) absence of *T. batrachorum*, (2) a weak saline solution (e.g., one-half strength Ringer's), (3) a piece of host rectum in the initial culture, (4) frequent addition of new nutrient materials which will act as substitutes for tissues (gastric mucin and Loeffler's dried serum together appear to satisfy this condition), (5) judicious additions of starch in the form of rice powder appear to be advantageous.

The initial partial success in cultivating *T. augusta* was perhaps associated with the presence of host tissues in the culture. Dried blood serum might be thought of as a good tissue substitute but it was not as effective as the combination with mucin. The latter apparently provided needed elements not available from the serum. One would suppose that beef heart and egg-meat media would be effective tissue substitutes but they did not seem to be as satisfactory as the serum-mucin combination.

The fact that *Trichomonas augusta* ingests starch so readily is interesting since it is a substance not likely to be present in the rectum of its host. Examination of tadpoles discloses that in these larval hosts, *T. batrachorum* is the more common species, while *T. augusta* is less common, and when found, is present in small numbers and is smaller in size. Yet tadpoles are supposed to be omnivorous and possibly more herbivorous than carnivorous. In adult frogs, on the other hand, which presumably are exclusively carnivorous, *Trichomonas augusta* is larger in size, has a high incidence and populations are often dense. *T. batrachorum* in adult hosts has a lower incidence and a lower density of population. Ordinarily *T. augusta*, when taken directly from the host does not contain solid food bodies, although its cytoplasm may be filled with small droplets of fluid (Kofoid and Swezy, 1915). *T. batrachorum*, by contrast, is an omnivorous feeder and commonly contains bacteria, *Blastocystis*, and other solid objects in its food vacuoles. Obviously, there is an intriguing problem of nutrition in relation to these two species that live together in amphibian hosts.

The maintenance of populations in cultures by adding nutrient materials and water suggests some interesting problems. One might suppose that very soon the accumulation of noxious waste products would inhibit or destroy the viability of the flagellates. The addition of mucin 30 times and dry serum 31 times within a period of 177 days, or an average of one addition every 3 days (culture A) might be expected to increase very greatly the concentration of osmotically active materials. Perhaps that is why this tube became negative. The necessity of discontinuing the cultures at the end of June, 1945, prevented the determination of the ultimate capacity of these flagellates to survive under the conditions provided.

#### SUMMARY

*Trichomonas augusta* is commonly found in great numbers in adult amphibian hosts and is often accompanied by *T. batrachorum* in much smaller numbers. When rectal contents of frogs containing these two species are placed in culture media usually *T. batrachorum* multiplies readily while *T. augusta* disappears within one or 2 weeks.

In my laboratory many attempts to cultivate *T. augusta* had failed. Partial success was attained when a piece of rectum containing this species was placed in modified Ringer's solution containing 0.2% gastric mucin.

Further trials showed that *T. augusta* may be cultivated under the following conditions: (1) absence of *T. batrachorum*; when this species is present it usually multiplies more rapidly than *T. augusta* and eventually overwhelms the latter; (2) use of a weak salt solution (e.g., one-half strength Ringer's); (3) addition of a piece of frog's rectum to the initial culture; (4) frequent addition (every 2 to 4 days) of some tissue substitute; alternate use of Loeffler's dried serum and gastric mucin was found to be successful; (5) judicious addition of starch in the form of powdered rice seems to be advantageous.

Under natural conditions, *T. augusta* appears to prosper better in adult amphibia while *T. batrachorum* seems to have the advantage in tadpoles. The latter species is omnivorous, feeding on bacteria and other organisms in the host's rectum. *T. augusta* seldom contains solid food bodies. Its avidity for starch, which it would not be expected to find in its carnivorous adult host, is somewhat surprising. Many problems of nutrition related to these two species of *Trichomonas* remain to be solved.

## REFERENCES

- DRBOHLAV, J. J. 1925 Une nouvelle preuve de la possibilité de cultiver *Entamoeba dysenteriae* type *histolytica*. Ann. Parasitol. hum. et comp. 3: 349-357.
- KOFOID, C. A. AND SWEZY, O. 1915 Mitosis and multiple fission in trichomonad flagellates. Proc. Am. Acad. Arts and Sci. 51: 287-378.
- MORGAN, B. B. 1944 Bovine trichomoniasis. Minneapolis, Burgess Pub. Co.
- ROSENBERG, L. E. 1936 On the viability of *Tritrichomonas augusta*. Trans. Am. Micr. Soc. 55: 313-314.
- WALTON, A. C. 1941 The Protozoa as parasites of Amphibia. List of Parasites. Contrib. Biol. Lab., Knox College, No. 76.



PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL *EUSTRONGYL-  
IDES*. VIII. INFLUENCE OF RESPIRATORY POISONS UPON  
THE AEROBIC GASEOUS METABOLISM

THEODOR VON BRAND<sup>1</sup>

Dept. of Biology, The Catholic University of America, Washington, D. C.

The larva of *Eustrongylides ignotus* which lives in cysts inside various fishes is a parasitic nematode that, contrary to many other endoparasites, leads normally a predominantly aerobic life. Two observations point in this direction. The first is that the blood-red color of the worms is due to haemoglobin dissolved in the body fluid (von Brand, 1937); the presence of such a concentration of this respiratory pigment could hardly be understood unless it were used to gather significant amounts of oxygen. The second observation represents an even more convincing argument. It was shown (von Brand, 1942) that a *Eustrongylides* exposed to an experimental anaerobic period accumulates an oxygen debt which is repaid over a period of several hours as soon as oxygen becomes again available. However, worms isolated from their cysts immediately prior to the determinations give very little evidence of having contracted there an oxygen debt; only during the first half hour is their oxygen consumption somewhat higher than the steady level reached after that time.

In view of this situation it seemed of interest to characterize the aerobic respiration of this organism further, and this was done by studying both the influence of respiratory inhibitors and stimulants. Some data were also gathered concerning the question whether anaerobic processes can supplement the failing aerobic respiration when the latter is inhibited by poisons. A study along these lines appeared the more desirable as so far very little is known about the nature of the aerobic respiration of parasitic nematodes. The only comparable investigation is that by Stannard, McCoy and Latchford (1938) who used the larvae of *Trichinella spiralis*.

MATERIAL AND METHODS

Only worms freshly isolated from *Fundulus heteroclitus* were used, and for every experiment new specimens were employed. As in the experiments reported upon previously in this series, from two to four, occasionally five, worms constituted an experimental lot. In most cases only the oxygen consumption was studied, but in some series the respiratory quotient was also determined. The respiration apparatus was the same Warburg apparatus used previously (von Brand, 1942, 1943); details of the experimental procedures (time of equilibration, rates of shaking, carbon dioxide determinations, etc) will be found in these papers. It will be sufficient to outline here the general procedure and to emphasize those points in which the present experiments differed from the earlier ones.

The worms were introduced immediately after isolation into the Warburg vessels containing 2 ccm of 0.85% NaCl solution, and their oxygen consumption was followed for two hours in order to establish their normal level of metabolism. As mentioned in the introduction, the oxygen consumption is above normal during the first half

Received for publication, August 8, 1945.

<sup>1</sup> The author is indebted to the Elizabeth Thompson Science Fund for a grant towards the purchase of the respiration apparatus used in this investigation.

hour. These values were consequently rejected when the initial rate of oxygen consumption was calculated.

At the end of the two hours the worms were transferred to new vessels containing the experimental solutions (in most cases 2 ccm of 0.85% NaCl solution plus various concentrations of poisons). This procedure was used in preference to adding the poisons through a sidearm in order to avoid having traces of the active substances distill over into the saline during the initial period. Many of the poisons used are not volatile, it is true, but it seemed preferable to use a uniform procedure in all cases. One drawback of the adopted method was that the vessels had to be reequilibrated to the temperature of the water bath before the first reading could be taken. Since this investigation is concerned with the maximal effect that a given substance exerts, the neglect of the first 20 minutes of exposure does not matter. The respiration within the experimental solutions was followed for two hours, in most instances. In some cases, however, in which in this time no noticeable effect had taken place, readings were continued for a total of 3 to 5 hours.

In the majority of those experiments that were of shorter duration, the worms were then transferred from the experimental solutions, after a thorough rinsing in 0.85% NaCl solution, to new vessels containing pure saline in order to test whether a recovery from the influence of the substances tested did take place. This period extended usually over 2 to 3 hours. At the end of these determinations the worms were removed from the Warburg vessels and kept over night in Erlenmeyer flasks containing saline, to give an opportunity of testing whether a permanent injury had taken place. In some series another determination of the oxygen consumption was carried out at that time (i.e., after 24 hours); it will be designated in the following sections as "second recovery period."

The temperature employed in all experiments was 41° C. It has been shown previously (von Brand, 1943) that the larvae are quite resistant against high temperatures in vitro, and it seemed desirable to have as high a metabolic rate as possible. It should be pointed out, furthermore, that the selected temperature is not unphysiological. The final hosts of the worms in question are herons, and, once the worms juveniles that have reached the definitive hosts. Birds, however, are generally characterized by their high body temperature.

#### EXPERIMENTAL RESULTS

1. *The influence of inhibitors on the oxygen consumption.*—We shall consider in the following paragraphs the influence of poisons that are commonly considered as inhibitors irrespective of whether or not they actually produced such an effect in the present case. The following substances fall into this category: potassium cyanide, sodium azide, sodium arsenite, sodium pyrophosphate, iodo-acetic acid and ethyl urethane.

a. *Potassium cyanide.*—The respiration of the worms proved to be fairly sensitive against this poison. Concentrations between M/100 and M/3200 brought about practically the same degree of inhibition, while it was somewhat less pronounced in M/6400 (Fig. 1). The mean value of the initial period, for the former range of concentrations, was  $109 \pm 4.2$  cmm  $O_2$ /gm/half hour; that of the KCN period  $31 \pm 1.5$ . The inhibition amounted, therefore, to 72%, while 28% of the respiration was cyanide resistant.

The worms themselves were not injured by the poison; they did not show any sign of damage, even after exposure to M/100 cyanide. In all cases the respiratory rate went back to a normal level. The time required for this recovery depended upon the cyanide concentration to which the worms had previously been exposed. In the stronger concentrations complete recovery did not occur during the 2 to 2½ hours of the first recovery period that followed immediately upon the exposure to the poison. But it was quite evident when the rate of oxygen consumption was determined after about 24 hours (second recovery period).

During recovery from the lower concentrations, distinct evidence of an excess oxygen consumption was found, i.e., the oxygen debt accumulated during the KCN period was at least partially repaid. This is clearest in the concentrations M/6400

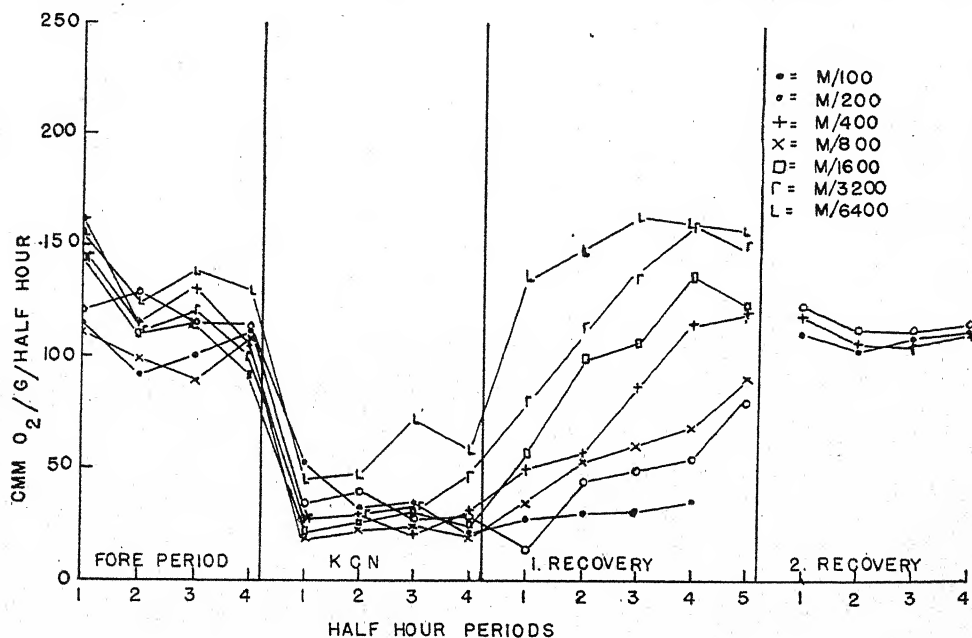


FIG. 1. Influence of various concentrations of potassium cyanide on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod, 1. and 2. recovery period: 0.85% NaCl; KCN period: specified concentrations of KCN dissolved in 0.85% NaCl. pH: 7; temperature: 41° C. Each point on the curves is the mean value of from 4 to 8 experiments.

and M/3200. The initial values for these series are 132 and 111 cmm O<sub>2</sub>/gm/half hour respectively. The total amounts of oxygen missed during the 2 hours experimental period are 304 and 308 cmm. The figures for the total oxygen consumption during the first recovery period are 765 and 645 cmm. Had the oxygen consumption been at its normal level during this time, 660 cmm or 555 cmm, respectively, would have been consumed; i.e., 105 cmm were consumed in excess in the first case, 90 cmm in the second. This corresponds to roughly 30% of the incurred debt in both cases.

It is obvious that these figures are only approximations. On the one hand, the oxygen debt was actually somewhat larger than indicated since the equilibration period was not taken into account. On the other hand, the oxygen consumption had not quite dropped to the original level at the end of the period during which recovery was studied; in other words, one can expect that during the following hours still

more of the incurred debt would have been repaid. Whether a complete repayment takes place is doubtful; it should be remembered in this connection that it is still incomplete after enforced anaerobiosis due to lack of oxygen in the surroundings (von Brand, 1942). This is due to the fact that the worms are capable of excreting at least parts of the non-oxidized products of the anaerobic metabolism (von Brand, 1938), and it appears reasonable to assume that only those products that actually accumulate in the tissues give rise to excess aerobic oxidations.

Whether a comparable repayment occurs in the higher concentrations has not been investigated. If it does, it must have occurred during the interval between first and second recovery period.

b. *Sodium azide*.—Sodium azide was tested in concentrations varying between M/400 and M/8000; the pH in these experiments was adjusted to 5.0, since it is

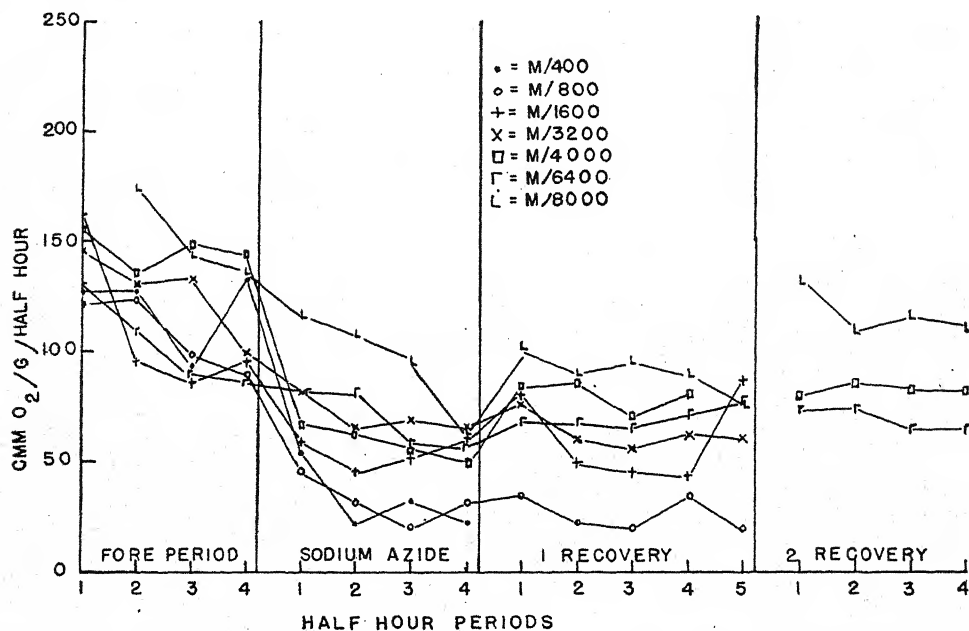


FIG. 2. Influence of various concentrations of sodium azide on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod, 1. and 2. recovery period: 0.85% NaCl; sodium azide period: specified concentrations of sodium azide dissolved in 0.85% NaCl. pH: 5; temperature: 41° C. Each point on the curves is the mean value of 4 experiments.

known that it exerts its most powerful influence in acid surroundings (Keilin, 1936). From a study of Fig. 2 it becomes apparent that the concentration of this poison is of much greater importance than in the case of cyanide. Maximal inhibition was observed only in M/400 and M/800 azide. The initial values of these two series were 117 and 103 cmm  $O_2$ /gmi/half hour; those for the sodium azide periods were 32 cmm in both cases. The maximal inhibition obtainable with this substance was therefore about 69 to 73%, values very close to those found in the KCN series. In the lower concentrations the inhibition was gradually less pronounced.

The blocking of the oxygen consumption due to azide was more permanent than that brought about by cyanide. The data summarized in Fig. 2 show that full recovery occurred only in the lowest concentration used (M/8000) and then only after



24 hours. It should be emphasized, however, that this curtailment in oxygen consumption did not seem to harm the worms in any way. They appeared perfectly healthy when the experiments were terminated and were then just as active as before.

c. *Sodium arsenite, sodium pyrophosphate, iodo-acetic acid and ethyl-urethane.*—None of these substances had a statistically significant influence upon the oxygen consumption of *Eustrongylides*. It is, therefore, hardly necessary to present the data in as detailed a form as for the two preceding poisons. The pertinent figures have been summarized in Table 1.

TABLE 1

Oxygen consumption of larval *Eustrongylides ignotus* under the influence of sodium arsenite, iodo-acetic acid, ethyl urethane and sodium pyrophosphate. Media: Foreperiod and recovery period: 0.85% NaCl; experimental period: specified poison dissolved in specified concentration in 0.85% NaCl. pH: 7; temperature: 41° C. The values presented for the various concentrations are the means of 4 experiments in each case. The values presented under "average" are the mean figures derived from all experiments carried out with one of the specified poisons.

Foreperiod	Experimental period			Recovery period	
cmm O <sub>2</sub> /gm/half hour	Concentration of poison	Time of exposure hours	cmm O <sub>2</sub> /gm/half hour	Duration hours	cmm O <sub>2</sub> /gm/half hour
I. Sodium arsenite					
120	M/100	4	135	Not studied	
106	M/500	4	123	" "	
117	M/1000	4	125	" "	
Average: 114 ± 5.8			126 ± 7.1		
II. Iodo-acetic acid					
127	M/800	2	119	2½	91
80	M/1000	2	99	2½	62
87	M/1600	2	95	2½	106
82	M/2000	2	84	2½	90
Average: 94 ± 4.9			99 ± 3.6		87 ± 5.2
III. Ethyl urethane					
100	M/5	2	99	2½	98
111	M/10	2	97	2½	105
84	M/20	2	76	2½	77
110	M/40	2	88	2½	91
Average: 101 ± 4.9			90 ± 3.6		93 ± 4.2
IV. Sodium pyrophosphate					
119	M/100	3	133	Not studied	
132	M/200	3	132	" "	
118	M/400	3	129	" "	
Average: 123 ± 5.0			131 ± 5.2		

The concentrations listed did not damage the worms. Sodium arsenite in a concentration of M/50 and M/500 iodo-acetic acid, however, killed a number of worms. No complete set of experiments could be performed with these concentrations and these data have consequently been omitted from Table 1. The lethal effect of the above two concentrations is nevertheless worthy of recording, since it indicated that at least sodium arsenite and iodo-acetic acid actually penetrate into the worms. Nematodes are covered by a strong cuticle and, if one gets negative results with a substance giving pronounced effects in other cases, the uncertainty remains as to whether it has passed into the tissues.

In so far as ethyl-urethane is concerned, a slight inhibition may have taken place. Although the differences are not statistically significant, one is tempted to assume that there has been an inhibition, because the value obtained during the experimental period was lower than those observed either during the initial or the recovery period.

2. *The influence of respiratory stimulants on the oxygen consumption.*—The following substances were tested: 2,4-dinitrophenol, para-phenylenediamine and methylene blue.

a. *Dinitrophenol.*—Dinitrophenol had a marked stimulatory effect on the oxygen consumption (Fig. 3). It was toxic in a concentration of M/250. The worms were still alive after the first recovery period, but, after being kept subsequently overnight in saline, a large percentage had died. An indication that the injury had taken place early can be deduced from the fact that the respiratory rate declined rather sharply during the first recovery period, in contrast to what was observed in the other concentrations. In the latter, the respiration remained high during the first recovery

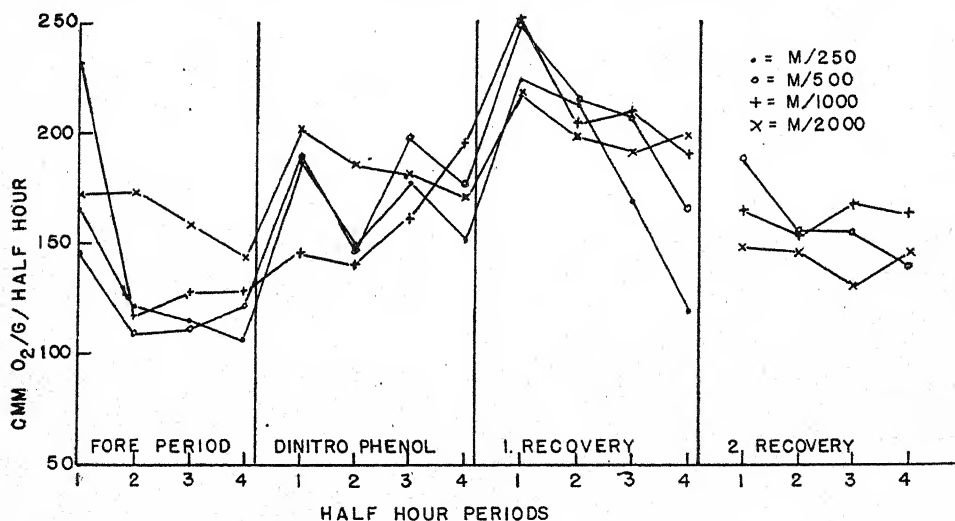


FIG. 3. Influence of various concentrations of 2,4-dinitrophenol on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod, 1. and 2. recovery period: 0.85% NaCl; dinitrophenol period: specified concentrations of dinitrophenol dissolved in 0.85% NaCl. pH: 7; temperature: 41° C. Each point on the curves is the mean value of 4 experiments.

period; as a matter of fact the level attained then was higher than either that of the initial or the dinitrophenol period. After 24 hours, however, the rate had dropped somewhat, but was still in excess of the normal values.

Although the variations between the different series were rather wide, no direct connection between the concentration and the rate of stimulation was apparent. In order to get a mean figure for the latter it appeared best, therefore, to average all the non-toxic concentrations (M/500 to M/2000). The following values in cmm O<sub>2</sub> per gram per half hour were obtained: Foreperiod:  $132 \pm 10.0$ ; dinitrophenol period:  $172 \pm 8.1$ ; first recovery period:  $207 \pm 13.2$ ; second recovery period:  $154 \pm 7.6$ . The average maximal stimulation (difference between foreperiod and first recovery period) was therefore 75 cmm O<sub>2</sub>/gm/half hour. This corresponds to about 57% of the normal respiration.

b. *Para-phenylenediamine.*—All the concentrations employed (M/100 to M/2000)

proved to be non-toxic. The results of these series are shown in Fig. 4. It is apparent that in all series a slight, but nevertheless distinct increase in the rate of oxygen consumption took place. There seems to be no connection between the degree of stimulation and the concentration of the active substance. It is therefore again justifiable to average the values of all series: initial oxygen consumption:  $117 \pm 5.4$  cmm  $O_2$ /gm/half hour; para-phenylenediamine period:  $140 \pm 5.3$ ; recovery period:  $131 \pm 4.9$ . The average excess oxygen consumption amounted consequently to 23 cmm per gram per half hour, or about 20% of the normal rate. The recovery value indicates that the influence of para-phenylenediamine is not as long lasting as that of dinitrophenol.

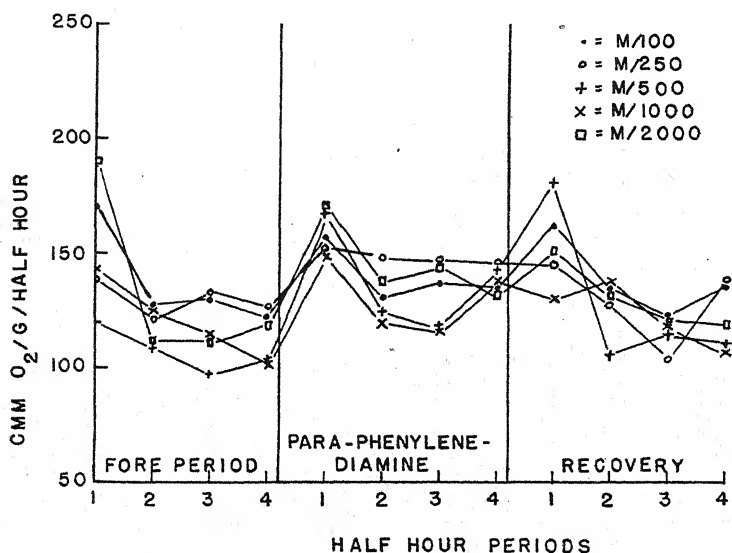


FIG. 4. Influence of various concentrations of para-phenylenediamine on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod and recovery period: 0.85% NaCl; p-phenylenediamine period: specified concentrations of para-phenylenediamine dissolved in 0.85% NaCl. pH: 7; temperature:  $41^\circ C$ . Each point on the curves is the mean value of 4 experiments.

It should be noted that para-phenylenediamine is occasionally responsible for a much greater increase in oxygen consumption than indicated by the above figures. In a single experiment, in which the concentration was M/250 and which was not used in drawing Fig. 4 or calculating the average figures, the initial oxygen consumption was 140 cmm per gram per half hour. It rose during the experimental period to 352 cmm and fell during recovery to 185 cmm. The stimulation amounted in this case to about 160%. No satisfactory explanation can be offered for this aberrant behavior. The only difference between this and the other experiments was that the worms employed were somewhat smaller than usual (49 mgm per worm, as against 60 to 100 mgm in the majority of the other experiments).

c. *Methylene blue*.—Only one concentration was tested, M/400. The initial value was 107 cmm  $O_2$ /gm/half hour, during the experimental period (5 hours duration) the oxygen consumption averaged 118 cmm. The similarity of both figures certainly excludes any large-scale stimulation.

There was no clear indication that the dye had actually penetrated the worms.

In the next series, therefore, the initial oxygen consumption was first determined as usual, but the worms were then cut in half and immersed into M/400 methylene blue. As controls, similar experiments were carried out in which the respiratory rate of cut worms was determined in saline. The following values were obtained: Foreperiod, methylene blue experiments: 122 cmm O<sub>2</sub>/gm/half hour; methylene blue period: 135 cmm. Foreperiod, saline experiments: 143 cmm; experimental period: 123 cmm. These values are again close, but nevertheless seem to support the idea that a slight stimulation may have been produced by the dye. It was certainly not much higher than 10% of the initial value.

3. *Influence of combination of poisons on the oxygen consumption.*—The series described in this section were designed to test in how far the increase in oxygen consumption that can be induced by various substances is cyanide sensitive. In the case of dinitrophenol and para-phenylenediamine the rate of oxygen consumption was

TABLE 2

Influence of cyanide on the oxygen consumption of larval *Eustrongylides ignotus* stimulated by para-phenylenediamine, dinitrophenol, methylene blue or potassium-chloride. Media: Foreperiod: 0.85% NaCl; experimental period: specified concentrations of para-phenylenediamine, dinitrophenol, methylene blue and their combinations with potassium cyanide in 0.85% NaCl. In the case of methylene blue the worms were kept prior to the first determination for 24 hours in M/400 methylene blue, dissolved in 0.85% NaCl; in that of potassium chloride, the worms were kept for a similar period in M/69 potassium chloride and the potassium cyanide used during the experimental period was in this instance dissolved in M/69 potassium chloride. pH: 7; temperature: 41° C. All the values presented are the means of 4 experiments. The duration of the experimental period was 2 hours in each case.

Foreperiod	Experimental period	
cmm O <sub>2</sub> /gm/half hour	Active substances employed	cmm O <sub>2</sub> /gm/half hour
123	M/250 p-phenylenediamine + M/1000 KCN	145
111		33
93	M/500 dinitrophenol + M/1000 KCN	181
92		35
Not determined	M/400 methylene blue + M/400 KCN	103
" "	M/69 potassium chloride + M/400 KCN	28
" "		147
		34

determined first for a period of 2 hours in the saline. This was followed in half of the experiments by a two-hour stay in the stimulating solution alone, while in the other half neutralized KCN was added to the stimulating solutions to bring the cyanide concentration to the strength specified in Table 2. Just as in the previous experiments, all these substances were prepared in a 0.85% NaCl solution.

A somewhat different procedure was used in the case of the potassium ion and methylene blue. It has been shown in a previous paper (von Brand, 1943) that potassium stimulates the oxygen consumption of *Eustrongylides* considerably when the worms are immersed into a KCl solution that is isotonic to 0.85% NaCl and that the increase is especially marked after a 24-hour stay in this solution. In the experiments reported here, therefore, the larvae were first kept for a day in a M/69 KCl solution and instead of an NaCl + KCN solution for the second half of the experiments a KCl + KCN solution was employed. Methylene blue was dissolved in 0.85% NaCl, but the animals prior to the determinations were kept for 24 hours in the dye solution in the desire to get as much methylene blue as possible into the worms.



The results of these experiments are summarized in Table 2. It is obvious that the level in oxygen consumption reached under the influence of cyanide was identical in all cases and closely corresponded to that induced by a pure cyanide solution (31 cmm, cf. a previous section). In other words, only the cyanide-sensitive part of the respiration was stimulated by dinitrophenol, para-phenylenediamine, the potassium ion and methylene blue, while the insensitive part remained entirely unaffected.

4. *Influence of poisons upon the carbon dioxide production and the respiratory quotient.*—In a number of experiments (Table 3), some of them identical with others mentioned in the preceding section, both carbon dioxide production and oxygen consumption were determined. The respiratory quotient during the initial period varied in the different series between 0.90 and 1.05. It has been shown (von Brand and Simpson, 1944) that only about 25% of the oxygen which the worms consume in sugar-free solutions at a comparable temperature can be attributed to the oxidation of stored carbohydrates. The relatively high values of the initial respiratory quotients must, in conformity with a previously expressed view (von Brand, 1942), be due to incomplete oxidations that proceed to some extent even at an oxygen tension of the atmospheric air.

The chief point of interest in the present experiments is whether, or to what extent, anaerobic processes are stimulated by the substances used. It is obvious that if the respiratory quotient rises significantly above 1.0 such processes must go on. The respiratory quotient alone, however, is not in itself a reliable index for the extent of the anaerobic processes, since its magnitude depends largely on the level of the oxygen consumption. Let us take two organisms with the respiratory quotient of 1.0 for the aerobic oxidations in both cases, but one consuming per hour 10 and the other 100 cmm oxygen. Let us further assume that both produce 50 cmm of carbon dioxide additionally from anaerobic processes. The overall respiratory quotient will be in the first case 6.0, but in the second only 1.5.

It was clear, therefore, that an attempt had to be made to calculate the actual carbon dioxide that originated on the one hand from the aerobic and on the other from the anaerobic metabolism. Consequently, the amounts of carbon dioxide originating aerobically during the experimental period were calculated under the assumption that they would have the same respiratory quotient as those of the initial period. This amount was then subtracted from the total carbon dioxide produced during the experimental period and the resulting figure was considered as "anaerobic carbon dioxide." The values thus obtained are probably not quite exact, since, as explained above, some incomplete oxidations are already present in the foreperiod, but there is no indication that their magnitude is of such an order as to vitiate the present calculation.

A survey of the figures presented in Table 3 show that para-phenylenediamine and iodo-acetic acid had no significant effect on the respiratory quotient. The very small amount of anaerobic carbon dioxide calculated for the latter poison is probably not real. Under the influence of potassium cyanide, on the other hand, a considerable evolution of anaerobic carbon dioxide took place, i.e., the worms were capable of supplementing the failing aerobic mechanisms through anaerobic processes. Of interest is also the influence of dinitrophenol. It stimulated not only the oxygen consumption, but it led also to a pronounced increase in aerobic fermentations. The increase in the respiratory quotient is relatively small, but this is due, as explained

TABLE 3

Influence of various poisons on the respiratory quotient of larval *Eustrongylides ignotus*. Media: Foreperiod: 0.85% NaCl; experimental period: specified poisons dissolved in 0.85% NaCl. pH: 7; temperature: 41° C. Length of foreperiod and of experimental period: 2 hours each. All the values presented are the means of 4 experiments.

Foreperiod			Experimental period					
mm O <sub>2</sub> /gm/half hour	mm CO <sub>2</sub> /gm/half hour	RQ.	Active substances employed	mm O <sub>2</sub> /gm/half hour	mm CO <sub>2</sub> /gm/half hour	RQ.	mm CO <sub>2</sub> derived from aerobic processes	mm CO <sub>2</sub> derived from anaerobic processes
96	89	0.92		M/1000 KCN	27	140	5.29	25
123	126	1.02	M/250 Para-phenylenediamine	145	142	0.98	148	0
92	86	0.93	M/500 Dinitrophenol	181	242	1.34	169	73
113	117	1.03	M/1000 Iodo-acetic acid	104	116	1.12	108	8
111	117	1.05	M/250 Para-phenylenediamine + M/1000 KCN	33	140	4.24	35	105
93	85	0.92	M/500 Dinitrophenol + M/1000 KCN	35	157	4.49	32	125
114	102	0.90	M/1000 Iodo-Acetic acid + M/1000 KCN	38	101	2.66	34	67

above, to the high rate of oxygen consumption. The carbon dioxide derived from anaerobic processes was actually about 2/3 of that produced under the influence of cyanide, that is when the aerobic oxidations are greatly restricted.

When para-phenylenediamine or dinitrophenol and cyanide were combined, the production of anaerobic carbon dioxide rose to the level reached under the influence of cyanide alone. The differences are clearly within the experimental errors.

A somewhat different picture was obtained when the combination iodo-acetic acid and cyanide was studied. The anaerobic carbon dioxide production was then only about 60% of that obtained in the cases mentioned above. This, of course, is due to the fact that iodo-acetic acid is a specific inhibitor of anaerobic processes. As explained in a previous section, the concentrations both of iodo-acetic acid and cyanide, as used in the present experiments, were non-toxic, if used alone. The combination of both substances, however, injured the worms rather rapidly. At the end of the 2-hour experimental period they appeared limp, although still able to move. But when kept, after the end of the determinations, overnight in pure saline, most worms had died. It follows that the elimination of a large part of the aerobic processes, for example through cyanide, does not interfere with the viability of the worms, probably because they can fill their energy needs vicariously through anaerobic processes. If, however, in addition the anaerobic energy production is partially interfered with, then the remaining mechanisms prove to be not powerful enough to keep the worms alive.

#### DISCUSSION

The respiration of the larva of *Eustrongylides ignotus* shows some similarities to that of the larvae of *Trichinella spiralis*, as described by Stannard, McCoy and Latchford (1938), but also distinct differences. Both organisms are tissue parasites and nematodes. They are both members of the same order (ENOPLIDA), but belong to different suborders (DIOCTOPHYMATA and TRICHINELLATA, respectively).

In both cases a marked and reversible sensitivity to cyanide was found. It was somewhat less pronounced in *E. ignotus*, since in the range M/100 to M/1000 the inhibition was 88 to 90% in *Trichinella* and about 70% in *Eustrongylides*. A noteworthy difference is that under the influence of cyanide no increase in anaerobic carbon dioxide production was observed in the former, while it was quite pronounced in the latter. *Trichinella*, therefore, shows no sign of a Pasteur reaction, *Eustrongylides* does.

Para-phenylenediamine stimulates the oxygen uptake of both organisms, while methylene blue either has no influence at all (*Trichinella*), or only a very slight one (*Eustrongylides*). The reaction of the two worms to arsenite is quite different. This poison in a concentration of M/100 inhibits the oxygen consumption of the former by 70 to 80%, while no inhibition whatever is seen in the latter. Whether a similar difference exists in respect to iodo-acetic acid remains to be seen; it may be noted that Stannard, McCoy and Latchford found a very pronounced inhibition of the oxygen consumption by means of iodo-acetamide.

A similarity between both worms, in which they differ from the alcoholic fermentation of yeast or muscle glycolysis, lies in the fact that iodo-acetic acid inhibits only slowly and incompletely the carbon dioxide production from anaerobic processes. This was found by Stannard, McCoy and Latchford in anaerobically conducted experiments, and confirmed in the present case where anaerobic processes induced by cyanide were studied.

Differences in the respiratory mechanisms of parasitic worms that are not very far apart in the taxonomic system, such as those described above, are of interest and should be an incentive for a comparative study of more forms. Aside from their theoretical interest, such investigations might in the future gain practical importance. It is well known that many worms show considerable differences in their reactions to anthelmintics. While so far, to the author's knowledge, a definite correlation between drug action and metabolic processes has not been established, it would appear quite possible that differences in the cellular enzymatic mechanisms lie at the root of these differences.

It has been shown that the oxygen consumption of *Eustrongylides* is inhibited by cyanide and azide but not by pyrophosphate. It is stimulated by para-phenylenediamine and the excess oxygen consumption induced by this latter substance is completely inhibited by cyanide. Exactly the same criteria apply to cytochrome oxidase (Keilin, 1929). It seems therefore very likely that a large part of the respiration is due to the Warburg-Keilin system.

There is very much less indication for the presence of aerobic dehydrogenases, since no marked inhibition could be achieved by means of urethane. In organisms operating largely on dehydrogenase systems, a considerable percentage of the oxygen consumption can be inhibited by these narcotics, in *Glaucoma*, for example, between 40 and 60% (Lwoff, 1934), in *Pelomyxa* between 36 and 65% (Pace and Belda, 1944).

No signs indicating a participation of glutathione or other substances containing -SH groups were found. Iodo-acetic acid and arsenite have been reported to interfere with the activity of these substances (Hopkins, Morgan and Lutwak-Mann, 1938; Lwoff, 1934), but they are quite without influence in the present case.

The enzymatic mechanism underlying the dinitrophenol stimulation is obscure. It is, however, remarkable that immersion into a solution of this substance leads not only to an increase in oxygen consumption, but at the same time to a marked appearance of anaerobic processes. A somewhat, but not exactly similar situation prevails in yeast. Dinitrophenol does not markedly change the oxygen uptake of *Saccharomyces cerevisiae*, but it stimulates to a considerable extent its aerobic fermentations (Pickett and Clifton, 1941).

#### SUMMARY

1. The oxygen uptake of the larval *Eustrongylides ignotus* is strongly inhibited by cyanide and azide, much less by urethane.
2. Pyrophosphate, iodo-acetic acid and arsenite do not interfere with its oxygen consumption.
3. The aerobic respiration is considerably stimulated by dinitrophenol, less by para-phenylenediamine and least by methylene blue.
4. Cyanide inhibits completely the increase in oxygen uptake due to dinitrophenol, para-phenylenediamine, methylene blue and the potassium ion.
5. Iodo-acetic acid and para-phenylenediamine have no influence upon the respiratory quotient. The latter rises, however, considerably under the influence of cyanide, indicating the presence of a Pasteur reaction.
6. Dinitrophenol stimulates not only the oxygen consumption, but also the aerobic fermentations.
7. Under the combined influence of dinitrophenol and cyanide or para-phenylene-



diamine and cyanide the same amounts of anaerobic carbon dioxide are produced as when cyanide alone is employed.

8. If a combination of iodo-acetic acid and cyanide is used, less anaerobic carbon dioxide is produced, but the action of iodo-acetic acid is less pronounced than in muscle glycolysis.

9. It is concluded that a large part of the respiration is due to an oxidase of the Warburg-Keilin type, that there is little indication for the presence of aerobic dehydrogenases. No signs of participation of substances with sulfhydryl groups were found.

## REFERENCES

- HOPKINS, F. G., MORGAN, E. G., AND LUTWAK-MANN, C. 1938 The influence of thiol groups in the activity of dehydrogenases II. *Biochem. J.* **32**: 1829-1848.
- KEILIN, D. 1929 Cytochrome and respiratory enzymes. *Proc. Roy. Soc. London, B* **104**: 206-252.
- 1936 The action of sodium azide on cellular respiration and on some catalytic oxidation reactions. *Proc. Roy. Soc. London, B* **121**: 165-173.
- LWOFF, M. 1934 Sur la respiration du cilié *Glaucoma piriformis*. *Compt. Rend. Soc. Biol. Paris* **115**: 237-241.
- PACE, D. M. AND BELDA, W. H. 1944 The effects of potassium cyanide, potassium arsenite and ethyl urethane on respiration in *Pelomyxa carolinensis*. *Biol. Bull.* **87**: 138-144.
- PICKETT, M. J. AND CLIFTON, C. E. 1941 Effect of selective poisons on utilization of glucose by yeast. *Proc. Soc. Exper. Biol. and Med.* **46**: 443-445.
- STANNARD, J. N., MCCOY, O. R., AND LATCHFORD, W. B. 1938 Studies on the metabolism of *Trichinella spiralis* larvae. *Am. J. Hyg.* **27**: 666-682.
- VON BRAND, T. 1937 Haemoglobin in a larval nematode. *J. Parasitol.* **23**: 225.
- 1938 Physiological observations on a larval *Eustrongylides*. *J. Parasitol.* **24**: 445-451.
- 1942 ——. II. The aerobic respiration. *Biol. Bull.* **82**: 1-13.
- 1943 ——. IV. Influence of temperature, pH and inorganic ions upon the oxygen consumption. *Biol. Bull.* **84**: 148-156.
- AND SIMPSON, W. F. 1944 ——. VII. Studies upon survival and metabolism in sterile surroundings. *J. Parasitol.* **30**: 121-129.

NOTES ON THE GAPEWORMS (NEMATODA: SYNGAMIDAE)  
OF GALLIFORM AND PASSERIFORM BIRDS  
IN NEW YORK STATE

FRANS C. GOBLE AND H. L. KUTZ

Game Research Center, New York State Conservation Department,  
Delmar, New York

The importance of the gapeworm (*Syngamus trachea*) as a parasite of young gallinaceous birds is common knowledge. Since Klee (1903) first associated an outbreak of gapeworms in pheasants with the high incidence of the parasites in a nearby rookery, considerable interest in the possibility of cross-infection between the gapeworms of poultry and those of perching birds has been evidenced.

A number of British investigators have surveyed passerine populations for gapeworms (Lewis, 1925, 1926; Elton & Buckland, 1928; Morgan, 1931; Campbell, 1935), and artificial transmission of syngami from starlings, rooks, blackbirds (*Turdus merula*) to chickens has been accomplished (Leiper, 1926; Taylor, 1928; Rice, 1929; Clapham, 1934, 1935; Morgan & Clapham, 1934).

In this hemisphere Cram (1930) has reported *S. trachea* from some passeriforms in Alaska; Walker (1886), Manter & Pinto (1928), and Webster (1943) have recorded observations on gapeworms in robins; Cuvillier (1932) artificially transmitted gapeworms of pheasant origin to the house sparrow, and Ripple (1941) infected chickens with gapeworms of robin origin. No extensive surveys of passeriform birds for information on the occurrence of gapeworms have been made in this country to our knowledge.

Almost every morphological character proposed for the division of the genus into species (Leiper, 1913; Chapin, 1925) has been questioned (Chapin, 1925; Lewis, 1928). There has been no treatment of all the avian parasites of the genus since Cram's (1927) which could not consider the status of *S. merulae* Baylis, 1926, or the taxonomic problems indicated by the work of Lewis (1928). It is the purpose of this article to record observations on the gapeworms we have encountered in galliform and passeriform birds and to discuss the identity of the parasites.

MATERIALS

The hosts examined for gapeworms are listed in Table 1. The galliforms were submitted to the laboratory at various times between 1938 and 1944. All of the birds listed were from the wild. The passeriform birds were collected by various means during the period of March through December 1944. Some were shot, some were trapped, others were victims of predation or accidental trauma. The species collected represent a haphazard sample of the common forms which occur in the vicinity of several game farms and refuges in upper New York state.

Birds listed as juveniles were those in which the *bursa fabricii* was still present. No passeriforms were collected after December, so probably most of the juveniles were less than 6 months old. Grouse and pheasants, however, were taken during all months of the year and since the bursa persists into the winter in these birds, some of those recorded as juveniles were as old as 9 months.

Received for publication, August 8, 1945.

Specimens of *Syngamus* collected from the tracheae of the listed species were fixed in hot 70 per cent alcohol with 5 per cent glycerin, and were examined either in glycerin, after the evaporation of the alcohol, or in lactophenol. The number of worms available for morphological study is indicated in Table 2. Abundant *Syngamus* material collected from artificially propagated pheasants from the State Game Farms was also available.

## OBSERVATIONS ON INCIDENCE

The incidence of gapeworms in the galliform and passeriform birds examined is indicated in Table 1. The generally accepted principle of greater susceptibility of younger animals to parasitism, is borne out by these data. It is obvious, however, that information from a survey of this type must be regarded as very general in nature, since the geographical origin of each infection in the migratory birds is usually uncertain and the incidence in the non-migratory game birds may depend greatly on the particular environment in which they are collected.

TABLE 1.—Occurrence of *Syngamus* in wild birds in New York

Hosts	Juveniles			Adults		
	Examined	Infected	Per cent	Examined	Infected	Per cent
Galliformes						
Ruffed Grouse ( <i>Bonasa umbellus</i> )	301	3	1	303	0	0
Ringnecked Pheasant ( <i>Phasianus colchicus</i> )	203	10	5	937	5	0.5
Passeriformes						
Eastern Crow ( <i>Corvus b. brachyrhynchos</i> )	12	3	25	25	0	0
Eastern Robin ( <i>Turdus m. migratorius</i> )	20	14	70	55	12	22
Eastern Meadowlark ( <i>Sturnella m. magna</i> )	8	2	25	16	1	6
Bronzed Grackle ( <i>Quiscalus versicolor</i> )	6	5	83	38	6	16

The following numbers of other birds have been examined for *Syngamus* and found negative: Hungarian partridge (*Perdix p. perdix*)—42; Eastern bluebird (*Sialia s. sialis*)—36; Starling (*Sturnus v. vulgaris*)—118; House sparrow (*Passer d. domesticus*)—50; Bobolink (*Dolichonyx oryzivorus*)—20; Eastern redbird (*Agelaius p. phoeniceus*)—39; Cowbird (*Molothrus a. ater*)—66; Slate-colored junco (*Junco h. hyemalis*)—22; Eastern tree sparrow (*Spizella a. arborea*)—20; Eastern song-sparrow (*Melospiza m. melodia*)—26.

Six of the 10 cases observed in juvenile pheasants occurred in a collection of 21 chicks taken in a limited area in the Lake Plains region. While the incidence there (6 out of 21, or 29 per cent) might be assumed from this collection, the figure of 5 per cent obtained from collection all over the state is likely to mean little in judging what infections might be expected in any one area. The 3 grouse which had gapeworms all occurred in the same covert in the southern Catskills and were, to the best of our judgment, all members of the same brood.

The absence of infections in adult crows and the incidence of 25 per cent in juvenile crows is in sharp contrast to the situation in the rook (*Corvus frugilegus*) in Britain, where Elton & Buckland found syngami in 4 out of 8 adults and 31 out of 33 juvenile rooks. It will be noted that no infections were observed in starlings although 118 (40 of which were juveniles) were examined. Lewis (1925, 1926) reported a gapeworm incidence of 35–37 per cent in this species in Britain and considered it to be an important distributor of gapeworms to poultry populations.

Since previous workers have not recorded the ages of the robins examined for

gapeworms it is difficult to compare our figures with theirs. Manter & Pinto (1928) reported gapes in 5 out of 7 examined; Ripple (1941) in 25 of 76 and Webster (1943) in 1 of 19. We have encountered no previous records of syngami in meadowlarks and bronzed grackles. Of the passeriform hosts from which Cram (1930) reported *S. trachea* in Alaska, we have had opportunity to examine only slate-colored juncos. No gapeworms were found in this species.

#### SIZE OF INFECTIONS AND PATHOLOGY

The numbers of worms encountered in infected birds is shown in Table 2. It is apparent that in most cases the juveniles harbored more parasites than the adults. There was no indication that either sex is more frequently or more heavily infected although the numbers we have examined may not be conclusive on this point. The

TABLE 2.—Numbers of worms encountered in *Syngamus* infections

Species of bird	Age of bird	Number of pairs of worms		
Ruffed Grouse	Juvenile	1 bird	had	1 pr.
		1 bird	had	2 prs.
		1 bird	had	3 prs.
Ringnecked Pheasant	Juvenile	4 birds	had	1 pr. each
		1 bird	had	2 prs.
		2 birds	had	3 prs. each
		1 bird	had	4 prs.
		1 bird	had	5 prs.
		1 bird	had	6½ prs.
	Adult	3 birds	had	1 pr. each
Eastern Crow	Juvenile	2 birds	had	2 prs. each
		3 birds	had	1 pr. each
Eastern Robin	Juvenile	1 bird	had	1 pr.
		2 birds	had	2 prs. each
		1 bird	had	3 prs.
		3 birds	had	4 prs. each
		1 bird	had	5 prs.
		2 birds	had	6 prs. each
		1 bird	had	7 prs.
		2 birds	had	8 prs. each
		1 bird	had	45 prs.
	Adult	10 birds	had	1 pr. each
Eastern Meadowlark	Juvenile	1 bird	had	2 prs.
		1 bird	had	3 prs.
		2 birds	had	1 pr. each
Bronzed Grackle	Adult	1 bird	had	1 pr.
		1 bird	had	lesions only
	Juvenile	2 birds	had	1 pr. each
		2 birds	had	2 prs. each
		2 birds	had	2 prs. each
		3 birds	had	1 pr. each
		2 birds	had	2 prs. each
	Adult	1 bird	had	3 prs.

birds which were regarded as adversely affected by their gapeworm infections are discussed in the following paragraphs.

In August, 1939, a grouse chick was found sick in the wild in the southern Catskills, was caught by hand and sent to the laboratory alive. Post-mortem examination revealed cachexia and the presence of 3 pairs of mature gapeworms, which occupied a large part of the trachea in this 179-gram bird. There was little food in the alimentary tract and no grit in the gizzard. When an investigator reached the locality in which the bird was found 12 days later and collected 2 more grouse, thought to be members of the same brood, it was found that both of the latter birds were also infected but neither showed any abnormal behavior.

In July, 1942, a pheasant chick, about 3 weeks old, was found dead on an area in Sussex County (Long Island) where population studies had indicated a high



brood mortality. Death of this bird was attributed to syngamosis; there was also a slight proventriculitis due to *Dispharynx* infection. Due to the paucity of the juvenile population only 2 other chicks were collected from the same area, 1 of which (4½ weeks old) harbored gapeworms and *Dispharynx*, the other (1½ week old) being negative. The 4½-week-old chick had a severe proventriculitis. Intensive working of the area in September resulted in the collection of 10 pheasants, all adults. It is unfortunate that subsequent surveys have been impossible, since circumstantial evidence indicated such a high brood mortality in the presence of parasitism.

One of the robins examined was found dead, obviously as the result of severe parasitism. In addition to the complete blocking of the trachea by 45 pairs of gapeworms, the bird harbored numbers of *Dispharynx*, *Capillaria*, *Porrocaecum* and *Dilepis*. Another young robin, which was captured by a cat, showed severe tracheitis associated with the presence of 8 pairs of gapeworms, but in another bird with the same number of worms no gross pathology was observed.

#### MORPHOLOGY AND IDENTITY OF THE WORMS

All of the gapeworms examined during this investigation were identified as *Syngamus trachea* (Montagu, 1811) Chapin, 1925, except those which occurred in the robins, which we consider to be *Syngamus merulae* Baylis, 1926. The latter is readily distinguishable from *S. trachea* on the basis of the size and cuticularization of the buccal capsule.

The forms regarded as *S. trachea* exhibited many of the variations which Lewis (1928) observed. Certain variations occurred, however, in our specimens of *S. trachea* from the bronzed grackle, which were not noted by Lewis in his study. In most cases the dorsal ray in the gapeworms from the grackle was divided in two for its full length. Sometimes both branches were simple, sometimes one of them was bifurcate. It is difficult to say whether this arrangement most resembles the pattern in Lewis' specimens from rooks, starlings or blackbirds. The form of the tail of the female gapeworms from grackles was consistent and differed from any described by other authors. In all cases there was a constriction or neck near the end of the blunt tail, giving the posterior part of the female body an appearance somewhat like a Folin-Wu Blood Sugar Tube.

#### DISCUSSION

Leiper (1913) suggested the following characters be used for differential diagnosis in the genus *Syngamus*: (1) relative position of buccal capsules in paired specimens; (2) length of the oesophagus relative to that of the body; (3) size and armature of mouth capsule; (4) relation of axis of buccal capsule to that of body; (5) outline of optical section of chitinous wall of capsule; (6) size of spicules; (7) configuration of posterior end of body of female; (8) site of excretory pore.

Chapin (1925), however, questioned the use of most of these above characters and proposed the use of the configuration of the bursal rays as characters of specific value. The value of the ray characters, however, as well as those involving the shape of the posterior end of the body of the female, were made doubtful by the work of Lewis (1928) who indicated very considerable variations in most of the characters previously used to distinguish species. In a series of SYNGAMINAE from galliform

and passeriform birds from the British Isles he recognized only *S. trachea* (in chickens, turkeys, bantams, pheasants, starlings and rooks) and *S. merulae* Baylis, 1926 (in blackbirds). He pointed out that the blunt tails of *S. parvus* and *S. gracilis* did not distinguish them from *S. trachea*, but that *S. parvus* might be differentiated on the basis of the lateral bursal rays.

Cram (1927), following Chapin, presented a key to the avian species of *Syngamus*, using dorsal ray characters and the length of the spicules. Clapham (1940) remarked that "identification of the species of the genus *Syngamus* from corvid birds presents no unusual difficulties." In the light of Lewis' work, however, which heavily discounts the use of the dorsal ray as a taxonomic character, difficulties are encountered in the use of the above-mentioned key and in the evaluation of the validity of some of the species of *Syngamus* found in birds.

Probably the most readily usable character is the presence or absence of a large cuticularized rim about the anterior edge of the buccal capsule. It is this character, in the main, which has enabled the separation of *S. merulae* from *S. trachea* almost at a glance (Baylis, 1926; Lewis, 1928; Clapham, 1934; present paper). The absence of the cuticularized expansion on the buccal capsule is characteristic of *S. merulae* Baylis, 1928, and *S. microspiculum* Skrjabin, 1915 (from Skrjabin's drawings). These two species have similar dorsal rays (Skrjabin, 1915; Baylis, 1926; Lewis, 1928; Manter & Pinto, 1928; Ripple, 1941) usually divided for half their length, each branch being bifurcate, sometimes trifurcate.

Certain discrepancies exist in Skrjabin's descriptions of *S. microspiculum* which make the length of the spicules in that form a matter of speculation. Skrjabin's earlier paper (1915) reported the spicule length to be 0.15 mm and it was stated that no other species of *Syngamus* had such small spicules, although (as Chapin noted) the spicules in *S. trachea* had been indicated, by numerous authors prior to that time, to be about 60  $\mu$ . In 1916 Skrjabin gave the length of the spicules in *S. microspiculum* as 0.115 mm. Since in the latter paper he also gave 0.69 mm as the spicule size in *S. trachea* (apparently intending 0.069 mm), Manter and Pinto (1928) suggested that the mistake lay in the position of the decimal point and that 0.0115 (11.5  $\mu$ ) was probably intended. By some calculation, inapparent to us, Clapham (1940) arrived at a figure of 49  $\mu$  for the spicules of Skrjabin's species.

That discrepancies in Skrjabin's figures are not always simply corrected by the moving of the decimal point has already been pointed out (Goble, 1941). Measurements on Skrjabin's drawing of *S. microspiculum* indicate that if the male body is 270  $\mu$  in diameter just in front of the bursa (as Skrjabin states) then the spicules are 88 to 90  $\mu$  long. This would appear to confirm the larger rather than the smaller measurements.

In describing *S. tenuispiculum* from the robin (*Turdus migratorius*) Manter and Pinto (1928) noted its similarity "to *S. merulae* Baylis (from *Turdus merula*, the blackbird) in size, buccal capsule, egg and spicules," but separated it on the basis of the dorsal ray. When Lewis' work appeared, they suggested (in a footnote, in press) that possibly their form would not be justified as a species separate from *S. trachea*. In 1941 Ripple conducted some experiments on transmission of the robin gapeworm to chicken and regarded *S. tenuispiculum* Manter and Pinto, 1928 as a synonym of *S. trachea*. Through the kindness of Dr. H. W. Manter we have been able to examine a pair of gapeworms from the group with which Ripple worked

and it is apparent that it is *S. merulae*. It is probable that all of his robin gapeworms were this species. We consider, therefore, that *S. tenuispiculum* is a synonym of *S. merulae*, not of *S. trachea*.

All species of avian syngami other than *S. merulae* and *S. microspiculum* appear to have heavily cuticularized expansions at the anterior edge of the buccal capsule. In this group are *S. trachea* (Montagu, 1811), *S. gracilis* Chapin, 1925 and *S. parvus* Chapin, 1925.

Chapin distinguished *S. gracilis* from *S. trachea* on the basis of the dorsal ray form, the inequality of the spicules, and the bent nature of the right spicule. Since the simple longitudinal splitting of the dorsal ray falls within the variations in ray pattern observed by Lewis and closely resembles the condition we have observed in gapeworms from bronzed grackles, this character is considered to be inadequate. Inequality of spicule length is quite common in the SYNGAMINAE, as is the bent condition of the right or longer spicule. Considering, therefore, that the characters proposed by Chapin are not qualitatively different from those typical of *S. trachea*, and that all recent collections of gapeworms from corvine birds both in Europe (Clapham, 1938, 1940) and North America (Beaudette, 1942; present paper) have been identified as *S. trachea*, it is regarded as probable that *S. gracilis* Chapin, 1925 is a synonym of *S. trachea* (Montagu, 1811).

The proper evaluation of the status of *S. parvus* Chapin, 1925 is difficult. Whether a spicule length of  $49\ \mu$  can be used to distinguish a species from another species where the range is  $53$  to  $82\ \mu$  is hard to say. In the light of what is known of variations in dorsal rays within this group, it is also difficult to judge the significance of the apparent stoutness and divergence of the lateral rays in *S. parvus*, in contrast to the slenderness and parallel arrangement in the other species of the genus. It is considered best to accord recognition to *S. parvus*, at least until material from either its type host, *Nucifraga caryocatactes*, or from other birds, becomes available for further study.

The following key includes the species of *Syngamus* occurring in galliform and passeriform birds. To our knowledge there is only one other avian species: described by El'perin (1938) from an owl (*Carine noctua*) in a Soviet publication which is unavailable to us.

- I. Buccal capsule *without* heavily cuticularized anterior rim (II).
  - A. Spicules longer than  $90\ \mu$  ..... *S. microspiculum*.
  - B. Spicules shorter than  $90\ \mu$  ..... *S. merulae*.
- II. Buccal capsule *with* heavily cuticularized anterior rim (I).
  - A. Spicules longer than  $50\ \mu$ . Lateral rays slender and parallel .... *S. trachea*.
  - B. Spicules shorter than  $50\ \mu$ . Lateral rays stout and divergent .... *S. parvus*.

#### SUMMARY

The incidence of gapeworms (genus *Syngamus*) in several species of galliform and passeriform birds in New York State is given. Records of *S. trachea* from meadowlarks and bronzed grackles are probably new. Data indicate significantly higher incidences in juveniles than in adults.

Observations were made on the size of infections and the pathological effects of the parasites on their hosts. The morphology and identity of the parasites were discussed. The form occurring in robins was considered to be *S. merulae* rather than *S. trachea*; the latter species occurred in all other species examined.

*S. tenuispiculum* Manter and Pinto, 1928 is considered to be a synonym of *S. merulae* Baylis, 1926. It is regarded as probable that *S. gracilis* Chapin, 1925 is a synonym of *S. trachea* (Montagu, 1811). A key to the species of *Syngamus* in galliform and passeriform birds is given.

## REFERENCES

- BAYLIS, H. A. 1926 A new species of the nematode genus *Syngamus*. *Ann. Mag. Nat. Hist.* 18: 661-665.
- BEAUDETTE, F. R. 1942 *Heterakis isolonche* Linstow (1906) in a pheasant with remarks on tuberculosis and gapeworms. *J. Am. Vet. Med. Assn.* 101: 274-275.
- CAMPBELL, J. W. 1935 The gapeworm (*Syngamus*) in wild birds. *J. Animal Ecol.* 4: 208-215.
- CHAPIN, E. A. 1925 Review of the nematode genera *Syngamus* Sieb. and *Cyathostoma* E. Blanchard. *J. Agr. Res.* 30: 677-681.
- CLAPHAM, P. A. 1934 Experimental studies on the transmission of gapeworm (*Syngamus trachea*) by earthworms. *Proc. Roy. Soc. London, B*, 115: 18-29.
- 1935 On the experimental transmission of *Syngamus trachea* from starlings to chickens. *J. Helm.* 13: 1-2.
- 1938 Are there host strains within the species *Syngamus trachea*? *J. Helm.* 16: 49-52.
- 1940 On the helminths of corvid birds in the British Isles. *J. Helm.* 18: 89-94.
- CRAM, E. B. 1927 Bird parasites of the Nematode suborders Strongylata, Ascaridata, and Spirurata. *U. S. Nat. Mus. Bull.* 140: 33-41.
- 1930 Gapeworm disease of birds in Alaska. *J. Parasitol.* 17: 56.
- CUVILLIER, E. 1932 Artificial infestation of the English sparrow, *Passer domesticus domesticus*, with the gapeworm, *Syngamus trachea*. *J. Parasitol.* 19: 93.
- EL'PERIN, M. A. 1938 Nova nematoda z rodu *Syngamus* Siebold—parazit trakhei sicha (*Athene noctua*). *Zbirn. Prats. Zool. Muz. Akad. Nauk. USSR* (21-22), *Trud. Inst. Zool. ta Biol.* 19: 197-204.
- ELTON, C. AND BUCKLAND, F. 1928 The gapeworm (*Syngamus trachea* Montagu) in rooks (*Corvus frugilegus* L.). *Parasitology* 20: 448-450.
- GOBLE, F. C. 1941 *Crenosoma zederi* n. sp. (Nematoda: Metastrongyloidea), a new lungworm from the skunk (*Mephitis mephitis*). *J. Parasitol.* 28: 381-384.
- KLEE, R. 1903 Krähen als Verbreiter von Geflügelseuchen. *Fortschr. d. Vet. Hyg.* 1: 43-44.
- LEIPER, R. T. 1913 Observations on certain helminths of man. *Tr. Roy. Soc. Trop. Med. and Hyg.* 6: 265-267.
- 1926 Gapes. *Proc. Zool. Soc. London. Part III*: 713-714.
- LEWIS, E. A. 1925 Starlings as distributors of "gapes." *J. Helm.* 3: 81-82.
- 1926 Starlings as distributors of "gapes." *J. Helm.* 4: 43-48.
- 1928 Observations on the morphology of *Syngamus* of some wild and domestic birds. *J. Helm.* 6: 99-112.
- MANTER, H. W. AND PINTO, H. E. 1928 A new species of gapeworm from the robin. *Tr. Am. Micr. Soc.* 47: 454-456.
- MORGAN, D. O. 1931 On the occurrence of gapeworms in nestling starlings and adult fowls. *J. Helm.* 9: 117-120.
- AND CLAPHAM, P. A. 1934 Some observations on gapeworms in poultry and game birds. *J. Helm.* 12: 63-70.
- RICE, J. P. 1929 The rook as a source of gapeworm infection. *J. Min. Agr. N. Ireland* 2: 84-87.
- RIPPLE, R. C. 1941 Studies on the gapeworm *Syngamus trachea* (Montagu, 1811) in robins and chickens. *J. Parasitol.* 27: 369-374.
- SKRJABIN, K. I. 1915 *Syngamus turkestanikih ptits.* *Vest. Obsh. Vet., Petrograd* 27: 645-658.
- 1916 Nematodes des oiseaux du Turkestan russe. *Ann. Mus. Zool. Akad. Imper. Sc., Petrograd* 20: 457-557.
- TAYLOR, E. L. 1928 *Syngamus trachea* from the starling transmitted to the chicken and some physiological variations observed. *Ann. Trop. Med. and Parasitol.* 22: 307-318.
- WALKER, H. D. 1886 The gapeworm of fowls (*Syngamus trachealis*). *Bull. Buffalo Soc. Nat. Sc.* 5: 251-265.
- WEBSTER, J. D. 1943 Helminths from the robin, with the description of a new nematode, *Porrocaecum brevispiculum*. *J. Parasitol.* 29: 161-163.



TWO NEW SPECIES OF ACARISCUS: *A. PLUVIUS* AND *A. ANOUS*  
(ACARINIDA: TROMBICULIDAE)

LIEUTENANT G. W. WHARTON, H(S) USNR<sup>1</sup>

U. S. Naval Medical Research Unit #2<sup>2</sup>

Two species of chiggers were found on sea birds collected at Guam. A plover and a noddy from Bougainville were infested with one of the species. The presence of chiggers on sea birds whose migrations are extensive may explain in part the wide distribution of certain trombiculids. The hosts of the species under discussion, plover, tattler, and noddy, are noted for long flights over open sea.

Both of these chiggers are typical of the genus *Acariscus* Ewing, 1943. The genera *Acariscus*, *Trombicula*, and *Eutrombicula* are closely related. *Acariscus* is more closely related to certain species of *Trombicula* than to *Eutrombicula*. However, since *Trombicula* has a three-pronged palpal claw, and *Acariscus* has a two-pronged claw, they are readily distinguishable. The line of demarcation between *Acariscus* and *Eutrombicula* is not so well defined. Both genera have the two-pronged palpal claw, but they are separated on the number of dorsal and ventral setae. *Eutrombicula* has 22 dorsal and 14 ventral setae. *Acariscus* has more than 22 dorsal setae and more than 14 ventral setae with at least two pairs posterior to the anus. The number of dorsal setae in some species is 22, while the number of ventral setae is more than 14. Womersley (1944), who has had more experience with Eastern chiggers than any other worker, does not accept *Acariscus* but includes it in his concept of *Trombicula*. The taxonomic system of the TROMBICULIDAE is still somewhat fluid even at the generic level. It is the author's opinion that additional genera will have to be raised, or sub-generic categories will have to be introduced so that relationships can be shown more exactly. It is for these reasons that the broad concept of *Trombicula* is not accepted and the present species are placed in *Acariscus*.

*Acariscus pluvius* n. sp.

(Fig. 1)

The first specimen of this species was found on a plover collected on Cape Torokina, Bougainville. Its name is a contraction of *Pluvialis*, the generic designation of its host. Later, additional specimens were taken from two species of noddies and a tattler. The species was not found on mammals or land birds although many of these were examined in areas adjacent to the beaches where the sea birds were taken. Only engorged or partially engorged specimens were collected.

*Body* (Fig. 1, A and B): The average length of five specimens was 470 microns; the average width 326 microns. The length and width vary with the stage of engorgement and are more a measure of the amounts of food ingested than they are specific characters. However, these measurements are significant in unfed and completely engorged specimens. The larvae are red in color. The striae are moderately developed anteriorly, but are weaker at the posterior end.

*Palps* (Fig. 1, C and D): Segment 1 has a branched seta originating in the anterior lateral region; it usually bears 6 to 8 barbs or branches. The single seta on segment 2 is stout and has about 12 branches. Segment 3 has a single seta that is nude or may have a single fine barb. The type specimen has a simple seta on the right and one with a single fine barb on the left. The dorsal, lateral, and ventral setae of segment 4 are all nude. Segment five has 7 barbed setae and

Received for publication, August 2, 1945.

<sup>1</sup> On military leave from Duke University.

<sup>2</sup> The opinions expressed are the author's and not necessarily those of the Navy Department.

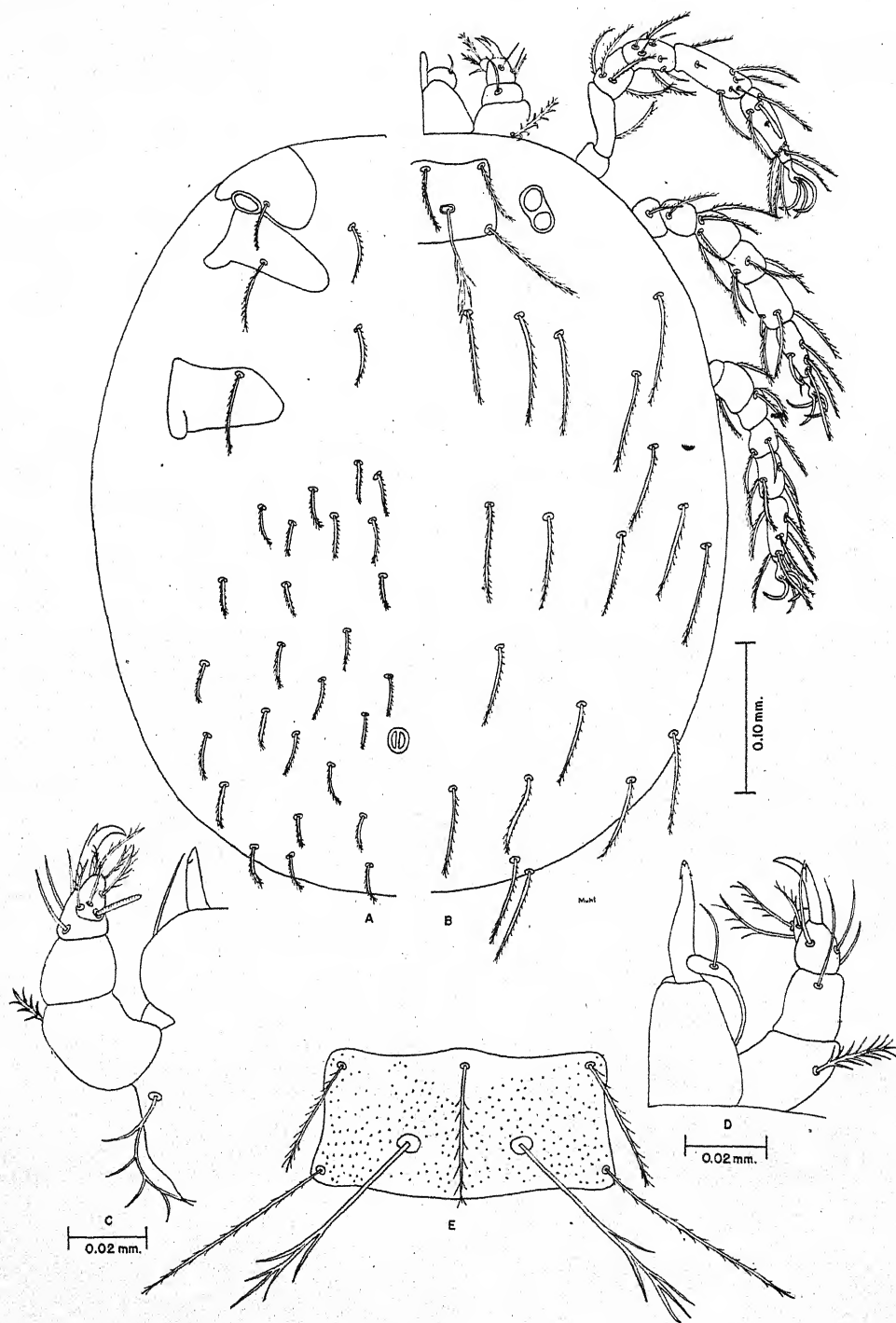


FIG. 1. *Acariscus pluvis* n. sp. A, ventral view; B, dorsal view; C, ventral view of capitulum; D, dorsal view of capitulum; E, scutum.  $\times 500$ .

one long sensory seta. The sensory seta is striated and similar in other respects to those of other species. The bifurcate palpal claw is long and slender, and its median element is the stoutest.

*Chelicerae* (Fig. 1, C and D): The basal segment is ornamented with a few small pits. It is about 37 microns long and 28 microns wide. The distal chitinous segment is strongly curved. The dorsal tooth is minute; the ventral tooth is small but recurved. The apex is sharp and points dorsally.

*Legs* (Fig. 1, A and B): The legs are of the usual form. There is a single seta on each coxa. Tarsi I and II are provided with a sensory seta.

*Scutum* (Fig. 1, E): The scutum is rectangular in shape. It is ornamented by pitting except in the area of the anterior-median seta. The setae are provided with numerous short barbs throughout their entire length. The pseudostigmata are simple pits. The pseudostigmatic organs that arise from them are filiform and provided with branches along the distal two-fifths. The average standard measurements (Wharton, 1945) in microns of five specimens follow: AW—68, PW—76, SB—28, ASB—27, PSB—18, AP—25, AM—39, AL—37, PL—65, S—59, DS—65.

*Setae*: Both dorsal and ventral setae are similar to the setae on the scutum. The sternal setae, however, have slightly longer barbs than the others. The arrangement of the setae in this species is not constant. The most usual dorsal formula is 2-10-10-6-6-2-2; however, the second row may have as many as 13 setae, while the fourth row may have as many as 8. During engorgement the setae shift so that formulae such as 2-10-10-6-5-4-2 or 2-13-9-7-4-2 are found. The ventral setae are even more variable in arrangement. They are not arranged in transverse rows but more nearly in longitudinal ones. Posterior to the 4 sternal setae are about 50 setae. The anus is situated slightly posterior to the center of the group.

*Hosts*: *Pluvialis dominica*, *Anous tenuirostris*, *Anous stolidus*, *Heteroscelus incanus*.

*Localities*: Cape Torokina, Empress Augusta Bay, Bougainville Island, Australian Mandate; Yapao Point, Guam, Mariana Islands.

*Type*: U. S. National Museum.

*Paratypes*: U. S. National Museum; South Australian Museum.

*Diagnosis*: *Acariscus pluvius* is more closely related to *Acariscus anous* than to any previously described species. Both species can be differentiated from their closest relative, *Acariscus gliricolens* (Hirst, 1915),<sup>3</sup> by their more numerous ventral setae.

*Acariscus anous* n. sp.

(Fig. 2)

Specimens of this species were found on sea birds from Guam. *Anous*, the generic name of the noddy, has been adopted for this mite. Unengorged as well as engorged specimens were taken from the birds.

*Body* (Fig. 2, A and B): The average length of five specimens is 420 microns; the width 320 microns. The type specimen is practically unengorged and measures 290 microns in length by 240 microns in width. The largest specimen measured was 560 microns by 410 microns. The larvae are red. The striae are well-developed anteriorly but are only moderately developed posteriorly.

*Palps* (Fig. 2, C and D): Segment 1 has a branched seta with about 6 barbs. The seta on segment 2 is stout with as many as 12 barbs. Segment 3 has a seta that bears one or two fine barbs. The dorsal lateral and ventral setae of segment 4 are nude. Segment 5 has seven branched setae and one extremely long sensory seta. The bifurcate palpal claw has a fine lateral and stout median element. The cheliceral shield bears a pair of nude setae.

*Chelicerae* (Fig. 2, C and D): The basal segment is lightly ornamented with a few pits. It is 48 microns long, 35 microns wide. The distal chitinous segment has a fairly well-developed ventral tooth, a sharp apex, and a minute dorsal tooth. It is strongly curved and points dorsally.

*Legs* (Fig. 2, A and B): There is a single branched seta on each coxa and a sensory seta on tarsi I and II.

*Scutum* (Fig. 2, E): The scutum is rectangular in shape. The ornamentation consists of numerous small pits that are more or less evenly distributed except in the region of the anterior-median seta where they are lacking. The pits are more numerous in this species than in *Acariscus pluvius*. The scutal setae are provided with short barbs along their entire length. The pseudostigmata are simple pits from which the distally branched, filiform pseudostigmatic

<sup>3</sup> New combination *Acariscus gliricolens* = *Trombicula gliricolens*, Womersley 1943 = *Microthrombidium gliricolens* Hirst 1915.

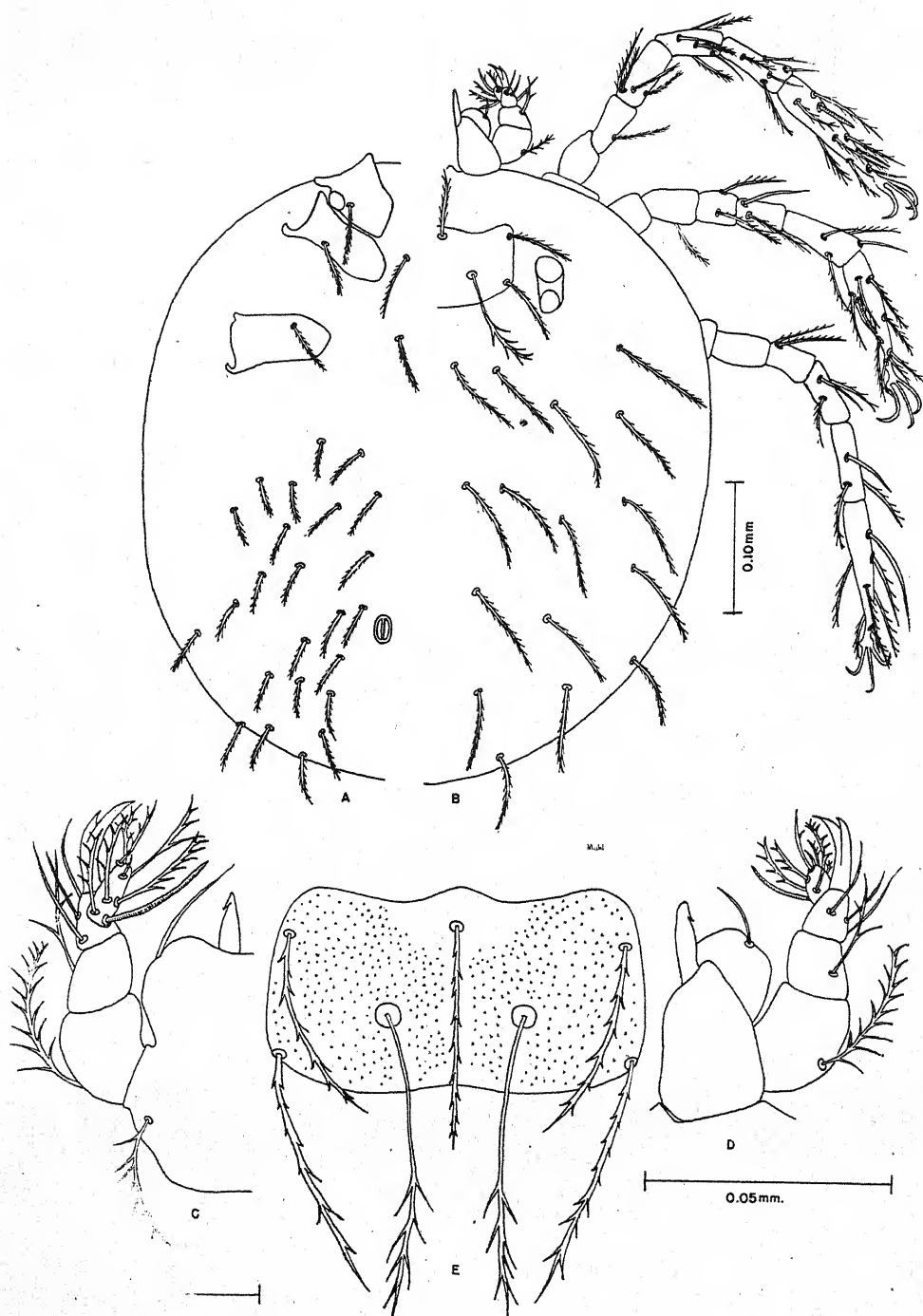


FIG. 2. *Acariscus anous* n. sp. A, ventral view; B, dorsal view; C, ventral view of capitulum; D, dorsal view of capitulum; E, scutum.  $\times 500$ .



organs arise. The standard measurements (Wharton, 1945) were determined for five specimens. The means of these data follow: AW—93, PW—101, SB—37, ASB—32, PSB—19, AP—30, AM—32, AL—53, PL—75, S—73, DS—75.

*Setae*: The dorsal and ventral setae are similar to the setae on the scutum; however, the ventral setae are shorter than the dorsal. The dorsal formula is usually 2-8-2-8-6-4-2. Posterior to the two pairs of sternal setae are about 50 ventral setae arranged more or less in rows. The anus is situated posterior to the center of the group of setae.

*Hosts*: *Anous stolidus*, *Heteroscelus incanus*.

*Locality*: Ypao Point, Guam, Mariana Islands.

*Type*: U. S. National Museum.

*Paratypes*: U. S. National Museum; South Australian Museum.

*Diagnosis*: *Acariscus anous* can be distinguished from *Acariscus pluvius*, its nearest relative, on the following characteristics: the longer sensory seta on the palpal thumb, the larger more densely pitted scutum, and the different and more constant arrangement of the dorsal setae.

#### REFERENCES

- EWING, H. E. 1943 The American chiggers (larvae of the TROMBICULINAE) of the genus *Acariscus*, new genus. Proc. Entom. Soc. Washington 45: 57-66.
- HIRST, S. 1915 On some new acarine parasites of rats. J. Econ. Biol. 10: 183-190.
- WHARTON, G. W. 1945 *Trombicula frittsi* n. sp. (ACARINIDA: TROMBICULIDAE). J. Parasitol. 31: 282-283.
- WOMERSLEY, H. 1944 Notes on and additions to the TROMBICULINAE and LEEUWENHOEKIINAE (ACARINA) of Australia and New Guinea. Trans. Roy. Soc. So. Australia 68: 82-112.
- WOMERSLEY, H. AND HEASLIP, W. G. 1943 The TROMBICULINAE (ACARINA) or itch-mites of the Austro-Malayan and Oriental regions. Trans. Roy. Soc. So. Australia 67: 68-142.

COMPARISON BETWEEN IN VITRO AND IN VIVO GLYCOGEN  
UTILIZATION IN THE FOWL NEMATODE  
*ASCARIDIA GALLI*<sup>1</sup>

W. MALCOLM REID<sup>2</sup>

Glycogen appears to be the most readily available source of stored energy in intestinal parasites. Investigators agree that one-third or more of the dry weight of many intestinal parasites consists of glycogen and it is known to disappear at a rapid rate under starvation conditions. Most available information on glycogen metabolism has come from in vitro studies. Weinland (1901), Schulte (1917), von Brand (1937a and 1937b) and Ro (1939) studied the nematode, *Ascaris lumbricoides*, while Toryu (1935) used *Ascaris megalocephala*. Ortner-Schönbach (1913) used histological methods, while Alt and Tischer (1931), von Brand (1933) and Wardle (1937) studied the cestode, *Moniezia*, by chemical methods. Von Brand found that 11 per cent of the total glycogen store was utilized in six hours. If this rate were to continue, two or three days would be required to reduce the glycogen to a trace, since the glycogen content was 2.69 per cent of the wet weight of the parasite at the time of removal from the host. Markov (1939), using similar chemical analyses to study larval cestodes of fish, found that 94 per cent of the initial glycogen of the plerocercoid stage of *Diphylllobothrium latum* was utilized in 72 hours at 35° C in Ringer-Locke's solution. The glycogen metabolism of the trematode, *Fasciola hepatica*, was investigated by Weinland and von Brand (1926). Lapage (1937), Wardle (1937) and Baldwin (1943) have criticized the use of an unbalanced one per cent sodium chloride culture solution in many of these experiments. Furthermore, the discovery that worms survive better with aseptic techniques (Glaser and Stoll, 1938; von Brand and Simpson, 1942), makes it desirable to reevaluate the earlier in vitro work.

The effects of in vivo starvation upon the glycogen content of the fowl cestode, *Raillietina cesticillus*, and of the fowl nematode, *Ascaridia galli*, were reported by Reid (1942, 1945). In one experiment with *A. galli*, females consumed 71 per cent of the stored glycogen in 24 hours while in another experiment the reserve was depleted 75 per cent in 48 hours. With *R. cesticillus* the rate of glycogen utilization was even higher, 94 per cent of the stored glycogen being utilized in 24 hours. Thus far no chemical studies of in vivo starvation of either mammalian cestodes or nematodes have been made. However, Hager (1941) noted reduction in the egg production of *Hymenolepis diminuta* after starvation of the rat host while Chandler (1943) reported inability of tapeworms of the same species to establish themselves or to grow to maturity in hosts on a restricted carbohydrate diet.

Were it possible to compare simultaneously the glycogen consumption under in vivo and in vitro methods, the value of the earlier experiments would be more readily assessed. Investigations have seldom been carried out in such a way as to make this

Received for publication, August 18, 1945.

<sup>1</sup> Part of the equipment for these studies was made available through an American Association for the Advancement of Science grant from the Illinois State Academy of Science.

<sup>2</sup> From the Department of Biology, Monmouth College, Monmouth, Illinois, and Marine Biological Laboratory, Woods Hole, Massachusetts.

comparison possible. Von Brand (1938) studied the tissue parasite, *Eustrongylides*, in this manner, but concluded that the metabolism of the species resembled that of free-living nematodes rather than intestinal parasites. Before an investigation using both methods upon a typical intestinal form has been completed, comparison between the high in vivo glycogen utilization found in the avian parasites and the lower in vitro glycogen consumption of larger mammalian forms can have relatively little significance. A comparison between in vivo and in vitro glycogen utilization in the avian *A. galli* has been undertaken in the present study.

#### MATERIAL AND METHODS

The methods used in the care of chickens and in parasitizing of them, together with the methods for glycogen determinations, were described elsewhere (Reid, 1945). With *R. cesticillus*, cessation of feeding of the host during the night resulted in a reduction of the glycogen level from 7.13 per cent of the wet weight of the worm at 6 PM to 3.92 per cent at 6 AM. This reduction indicated that the available carbohydrate in the gut was exhausted soon after feeding had ceased. Since the fowls had an abundance of commercial mash, mixed for battery-reared chicks, before them at all times, they fed frequently but intermittently and thereby stored very little feed in their crops. The contents of the intestine diminished gradually, beginning two to three hours after removal of feed from the pens and the upper gut was almost entirely empty after the normal fast during the night. Accordingly, in the present experiment feed was removed at 6 PM and the fowls were killed 50 hours later at 8 PM. This resulted in approximately 48 hours of worm starvation although in some cases carbohydrate from the gut may have been available for a slightly longer period.

For the in vitro studies, one per cent salt solution as a medium for study of intestinal parasites has been criticized by Lapage (1937) and others. However, since it was desired to compare and assess the results obtained by other investigators, methods similar to those of von Brand (1937a) were used. Worms were washed in saline after removal and blotted dry on filter paper. In order later to have sufficient weight for the analytical methods, one female or two males were placed in individual weighed Erlenmeyer flasks which contained warm one per cent saline. The worms were weighed and placed in an incubator at  $41.5^{\circ} \pm 1^{\circ} \text{C}$ , the temperature corresponding to the mid-afternoon temperature in the jejunum of fowls 57 days in age. Another criticism of the method commonly used with in vitro studies has been the maintenance of worms in an anaerobic environment. Slater (1925) concluded that *A. lumbricoides* became inactive and could not carry on normal activities when oxygen was excluded by the use of a hydrogen stream. However, since most other investigators have maintained their cultures under anaerobic conditions by this means, a hydrogen stream was passed through the flasks during the period of the present experiments. Although no attempt to bring about aseptic conditions was made, the saline was changed at the end of 24 hours. At the end of 48 hours worms were discarded from flasks which contained a nematode not responding to tactile stimulation while living worms were analyzed for glycogen.

#### EXPERIMENT

Chemical analyses were made on three groups of worms. Group I consisted of controls, group II of worms which had been starved within the host for 48 hours and

group III which had been starved in saline for 48 hours. Fowls, 59 days old, which had been parasitized with 300 embryonated eggs of *A. galli* 48 days before, were used as a source of mature worms. After killing some of the chickens the worms were recovered, separated by sexes and divided into two lots. One half of both the males and the females, used for group I controls, were placed immediately in potassium hydroxide for glycogen analysis. The other half, as group III, were weighed in warm saline and placed in the incubator for 48 hours. At the end of this period the parasites were killed for glycogen analysis. Group II worms were provided by depriving other fowls of feed for 50 hours and then removing and killing the nematodes under conditions similar to those of groups I and III.

The results of glycogen analyses from worms of the three groups are summarized in Table 1. In females, the glycogen store was reduced from 4.66 to 1.16 per cent after 48 hours of in vivo starvation while in vitro starvation resulted in reduction to 1.01 per cent. In males the glycogen store was reduced from 3.81 to 0.43 per cent under in vivo and 0.26 under in vitro conditions. Both males and females showed slightly greater glycogen utilization under in vitro than under in vivo conditions, the difference being 0.17 in males and 0.15 in females. Considering the difficulty in determining the onset of in vivo starvation and other variables inherent in the biological material used, these results show a remarkable likeness in glycogen utilization under the two conditions in the experiment.

TABLE 1.—Glycogen utilization in *Ascaridia galli* during 48 hours of in vitro or in vivo starvation

Treatment	Sex and number of worm samples analyzed	Mean sample percentage of glycogen of the worm wet weight	Percentage of glycogen store utilized in 48 hours
Group I Unstarved controls	10 F	4.66 ± 0.30* (3.04-5.78) †	
	9 M	3.81 ± 0.07 (3.55-4.09)	
Group II In vivo starvation 48 hours	10 F	1.16 ± 0.10 (0.64-1.69)	75%
	10 M	0.43 ± 0.03 (0.12-0.82)	89%
Group III In vitro starvation 48 hours	8 F	1.01 ± 0.10 (0.72-1.44)	78%
	12 M	0.26 ± 0.03 (0.13-0.50)	93%

\* Standard error. † Numbers in parentheses denote range.

#### DISCUSSION

The close similarity in glycogen utilization between parasites within the host and those in one per cent saline indicates that the results obtained by in vitro work of other investigators probably give a fair picture of this phase of metabolism within the host. Since a slightly greater amount of glycogen was used under in vitro than in vivo conditions, this conclusion seems more fully substantiated. It appears probable that neither the media used nor the anaerobic conditions introduced by using a hydrogen stream materially affected glycogen utilization in these earlier experiments. Furthermore, the short survival period of parasites noted by all investigators under these conditions was not due to an upset in the normal glycogen metabolism.

Comparisons between the mammalian *A. lumbricoides* and the avian *A. galli* are now possible. Von Brand (1937a) found that *A. lumbricoides* females consumed



45 per cent of the total glycogen in 48 hours while males used 49 per cent in the same period. Ro (1939), using the same species, obtained somewhat lower figures. Females used 38 per cent and males 23 per cent in 48 hours. In avian parasites the glycogen store was utilized more rapidly; 78 per cent in females and 93 per cent in males during the same period. Among the factors responsible for this more rapid metabolic rate may be mentioned the smaller size of *A. galli* which averaged 0.080 gram in females and 0.035 gram in males while in von Brand's experiments *A. lumbricoides* females weighed 4.14 grams and males 1.13 grams. Another contributing factor was the higher temperature with the avian parasite, 41.5° C, as compared with 37° in von Brand's experiments.

*Ascaridia galli* are so dependent upon a carbohydrate food source that most of them are expelled after 96 hours of host starvation (Reid 1945). It seems likely that *A. lumbricoides* could also be affected profoundly by host starvation, but the starvation period would need to be longer. Various commercial handling techniques of fowls may result in sufficient starvation to induce natural loss of parasites. It remains to be determined whether or not this is the case with mammalian forms. However, it appears probable that the differences in the normal glycogen content of *A. lumbricoides* found by various investigators in different countries as noted by von Brand (1937a) could be accounted for by differences in treatment of the host before slaughtering.

#### SUMMARY

1. Both male and female *Ascaridia galli* utilized approximately the same amount of glycogen during 48 hours of in vivo starvation as under anaerobic in vitro starvation in one per cent sodium chloride. Females consumed 75 per cent of their total glycogen store when starved within the host as compared to 78 per cent when removed and kept in the incubator. Males consumed 89 per cent when starved in the host gut and 93 per cent in saline.

2. Since glycogen utilization under the two different conditions in these experiments was similar, it may be concluded that the in vitro technique for study of glycogen metabolism probably has reflected satisfactorily the conditions in vivo.

3. Comparison of the glycogen metabolism in the avian *A. galli* in the present experiments with the mammalian *Ascaris lumbricoides* studied by other investigators indicated that the smaller parasite found in the avian host apparently utilized the glycogen reserve much more rapidly than did *A. lumbricoides*.

#### REFERENCES

- ALT, HOWARD L. AND TISCHER, OTTO A. 1931 Observations on the metabolism of the tapeworm, *Moniezia expansa*. Proc. Soc. Exper. Biol. and Med. 29: 222-224.
- BALDWIN, ERNEST 1943 An in vitro method for the chemotherapeutic investigation of anthelmintic potency. Parasitology 35: 89-111.
- CHANDLER, ASA C. 1943 Studies on the nutrition of tapeworms. Am. J. Hyg. 37: 121-130.
- GLASER, R. W. AND STOLL, NORMAN R. 1938 Sterile culture of the free-living stages of the sheep stomach worm, *Haemonchus contortus*. Parasitology 30: 324-332.
- HAGER, ANNE 1941 Effects of dietary modification of host rats on the tapeworm *Hymenolepis diminuta*. Iowa State Coll. J. Sc. 15: 127-153.
- LAPAGE, GEOFFREY 1937 Nematodes parasitic in animals. 172 p. Methuen, London.
- MARKOV, G. S. 1939 Nutrition of tapeworms in artificial media. Compt. Rend. Acad. Sc. URSS (Dok.) 25: 93-96.
- ORTNER-SCHÖNBACH, PAULINE 1913 Zur Morphologie des Glycogens bei Trematoden und Cestoden. Arch. Zellforsch. 11: 413-449.

- REID, W. M. 1942 Certain nutritional requirements of the fowl cestode *Raillietina cesticillus* (Molin) as demonstrated by short periods of starvation of the host. *J. Parasitol.* 28: 319-340.
- 1945 The relationship between glycogen depletion in the nematode *Ascaridia galli* (Schränk) and elimination of the parasite by the host. *Am. J. Hyg.* 41: 150-155.
- RO, MANTOKU 1939 Anaerobic glycogen consumption in *Ascaris* females and males. *Act. Japon. Med. Trop.* 1: 29-36.
- SCHULTE, HUBERT 1917 Versuche über Stoffwechselvorgänge bei *Ascaris lumbricoides*. *Pflüger's Arch.* 166: 1-44.
- SLATER, WILLIAM KERSHAW 1925 The nature of the metabolic processes in *Ascaris lumbricoides*. *Biochem. J.* 19: 604-610.
- TORYU, YOSHIYUKI 1935 Contributions to the physiology of the *Ascaris*. III. Survival and glycogen content of the *Ascaris Ascaris megaloccephala* Colq. in the presence and absence of oxygen. *Science Repts. Tōkoku* 4 Ser. 10: 361-375.
- VON BRAND, TH. 1933 Untersuchungen über den Stoffbestand einiger Cestoden und den Stoffwechsel von *Moniezia expansa*. *Ztschr. Vergleich. Physiol.* 18: 562-596.
- 1937a The anaerobic glycogen consumption in *Ascaris* females and males. *J. Parasitol.* 23: 68-72.
- 1937b The aerobic resynthesis of glycogen in *Ascaris*. *J. Parasitol.* 23: 316-317.
- 1938 Physiological observations on a larval *Eustrongylides* (Nematoda). *J. Parasitol.* 24: 445-451.
- AND SIMPSON, W. F. 1942 Physiological observations upon larval *Eustrongylides*. III. Culture attempts in vitro under sterile conditions. *Proc. Soc. Exper. Biol. and Med.* 49: 245-248.
- WARDLE, ROBERT ARNOLD 1937 The physiology of the sheep tapeworm, *Moniezia expansa* Blanchard. *Canad. J. Res. Sec. D* 15: 117-126.
- WEINLAND, ERNST 1901 Über kohlehydratzersetzung ohne Sauerstoffaufnahme bei *Ascaris*, einen tierischen Gärungsprozess. *Zschr. Biol.* 42: 55-90.
- AND VON BRAND, THEODOR 1926 Beobachtungen an *Fasciola hepatica*. *Ztschr. Vergleich. Physiol.* 4: 212-285.

DERMADENA LACTOPHRYSI N. GEN., N. SP. (TREMATODA:  
LEPOCREADIIDAE) AND CONSIDERATION OF THE  
RELATED GENUS PSEUDOCREADIUM\*

HAROLD W. MANTER  
University of Nebraska

Among trematodes collected from marine fishes at the Biological Laboratory of the Carnegie Institution at Tortugas, Florida, in 1930-1932, is a distome from trunk-fishes, remarkable because of conspicuous glands with pores opening on the ventral surface. These glands are similar to those of certain monostomes (NOTOCOTYLIDAE). The study of these specimens involved consideration of *Distomum lamelliforme* Linton, 1907, from *Balistes* in Bermuda. The type specimen of *D. lamelliforme* was loaned by the U. S. National Museum through the kindness of Dr. E. W. Price. Numerous specimens of this same trematode collected in Bermuda by the late Dr. F. D. Barker were made available through the kindness of the General Biological Supply House. It is evident that Linton included material of two species under the name of *D. lamelliforme*. The type material, from *Balistes*, belongs in the genus *Pseudocreadium* Layman, 1930, while the specimens from *Lactophrys* evidently agree with my material from that same host at Tortugas and are referred below to a new species and genus of the LEPOCREADIIDAE closely related to the genus *Pseudocreadium*.

*Dermadena lactophrysi* n. gen., n. sp.  
(Figs. 1-8)

*Synonym:* *Distomum lamelliforme* Linton, 1907, in part.

*Description:* Body circular or subcircular in outline; with edges inrolling ventrally except at anterior end; provided with cuticular scales, especially anteriorly, which may be lost; sometimes wider than long; length 0.765 to 1.822 mm, width 0.735 to 1.917 mm. A specimen 0.690 mm long was immature, one 0.765 mm long contained two eggs. Pigment spots present on youngest specimens, indicating an oculate cercaria. Ventral surface of body with numerous pores located on more or less elevated papillae; each pore is the opening of a conspicuous, multicellular gland; leading to the pore is a definite duct surrounded by radially arranged, vacuolated cells enclosed in a fibrous wall; vitelline cells frequently invade the basal portions of glands so that clusters of vitelline cells indicate the location of most of the glands. Number of glands from about 20 to over 90, arranged roughly in concentric rings or rows; number of rings increasing with body size (Figs. 2-4); five glands in acetabular region, two at anterior border of acetabulum, three directly over the gonads. Oral sucker subterminal, spherical or subspherical, 0.073 to 0.170 mm in transverse diameter; acetabulum near middle of body in immature specimens but somewhat anterior to midbody in older specimens; slightly larger than oral sucker, 0.097 to 0.197 mm in transverse diameter; sucker ratio 1:1.12 to 1.3.<sup>1</sup> Prepharynx lacking, pharynx subspherical, sometimes wider than long, 0.059 to 0.085 mm long by 0.044 to 0.107 mm wide; short esophagus; ceca narrow, distant from edges of body, with short diverticula or outpocketings, curving around gonads, ends usually not far apart. Genital pore slightly to the left, at midpharynx level. Testes two, symmetrical, intercecal, separated by ovary, immediately posterior to acetabulum, smooth or irregularly crenulated. Cirrus sac elongate, clavate, extending from genital pore diagonally backward to pass slightly beyond the right anterior edge of

Received for publication, August 17, 1945.

\* Studies from the Zoological Laboratories, University of Nebraska, No. 224.

<sup>1</sup> In descriptions of trematodes, the ratio of the oral sucker to acetabulum is often expressed as 2:3, 3:4, 4:5, etc. It is proposed here to express this ratio with the value of the oral sucker always taken as 1. The result will be actually an acetabular index which can be directly compared with that of other trematodes (for example, 1:0.88 and 1:2.16). Another advantage is the expression of exact range of variation in sucker ratios.

acetabulum. Cirrus sac contains a basal, spherical, seminal vesicle; a prostatic vesicle of two parts each with large cells, the anterior part with more conspicuous nuclei; and a large cirrus, wider posteriorly, armed with short, wide tubercles or papillae. External seminal vesicle present. Ovary deeply trilobed, immediately posterior to acetabulum, between testes. Uterus short, pre-ovarian, with few eggs; seminal receptacle present to left of acetabulum near metraterm; metraterm well developed, muscular, extending along left side of cirrus sac but diverging slightly posteriorly, about same length as cirrus sac, not overlapping acetabulum. Vitelline follicles widely distributed in dorsal portion of body from level of intestinal bifurcation to near posterior end of body, filling most of the wide extracecal fields, contiguous posterior to testes but not anterior to acetabulum. Eggs thin-shelled, 60 to 68  $\mu$  long by 34 to 49  $\mu$  wide. Excretory pore dorsal, conspicuous, with radial cells, relatively far anterior, not far posterior to ovary; excretory vesicle appears to be Y-shaped but the arms connect with the short stem by a narrow constriction (Fig. 8) and probably represent enlarged collecting vessels.

*Hosts:* *Lactophrys tricornis* (Linn.), trunkfish; present in 7 of 25 hosts examined.

*Lactophrys trigonis* (Linn.), trunkfish; in 9 of 11 hosts examined.

*Lactophrys triqueter* (Linn.), trunkfish; type host, in 2 of 4 hosts examined.

*Location:* Intestine.

*Locality:* Dry Tortugas, Florida.

*Type specimen:* U. S. Nat. Mus. Helminthol. Coll., No. 36931.

*Diagnosis of the genus Dermadena:* Family LEPOCREADIIDAE. Characters of the genus *Pseudocreadium* Layman, 1930, together with the possession of conspicuous ventral glands. Body with evanescent spines, subcircular, flattened, with ventrally inrolling edges. Testes symmetrical, ovary intertesticular; cirrus sac clavate, with basal seminal vesicle, bipartite prostatic portion, and tuberculated cirrus; external seminal vesicle present; cirrus sac and metraterm not extending posterior to acetabulum; seminal receptacle large, anterior to ovary; excretory pore dorsal, not far posterior to ovary; excretory vesicle often appearing Y-shaped, the lateral arms, however, probably enlarged collecting tubes extending between ovary and testes. Type species: *Dermadena lactophrysi*.

The generic name is from *derma* = skin, and *adena* = gland, referring to the characteristic ventral glands. The species name is for the host.

*Discussion.*—This trematode is like species of *Pseudocreadium* Layman, 1930 (= *Hypocreadium* Ozaki, 1936; *Leptocreadium* Ozaki, 1936) except for the ventral glands. Several species of *Pseudocreadium* possess the same circular body with inrolled edges and the same details of cirrus sac and female organs. The ventral glands are so striking and unusual that the new genus is based on them.

The smallest specimen, about 0.5 mm in length and immature, possessed about 20 very distinct, prominent glands (Fig. 2). The number of glands increases with age (Figs. 2–4) until in one of the largest specimens over 90 could be counted. The outermost glands are smallest and least developed. Most of the glands are over the area occupied by the vitellaria; they are not irregularly scattered, as might appear at first glance, but are arranged in more or less regular rows or rings approximately parallel with the body outline. Large specimens possess five or six, more or less concentric rows, while the smallest had three. There is always a pair of glands at the right and left anterior edge of the acetabulum, a large gland over each testis, and one over the ovary. These five glands in the acetabular region develop early. Just median to the ceca is a ring of 10 or 11 glands. Single glands on each side of the excretory vesicle do not seem to be a part of a regular row unless they be considered to form with the three glands over the gonads a central, complete ring of five. In the 0.5 mm specimen the first extracecal row of glands was barely beginning to form; in a 0.75 mm specimen it had 16 glands; in a 1.376 mm specimen it had 19 glands while a second extracecal row had 22 and a third extracecal row approximately 24. Thus, the innermost (that is, more median) glands seem to be constant in number and position but the outer rows are somewhat irregular, varying in number and with some glands not exactly in alignment. It is clear that additional outer rows of glands develop, and probably a few glands may be added to the extracecal rows anteriorly.



The glands apparently may be protruded to varying degree. When protruded, the tip is narrowed and the entire elevation is nipple-shaped (Fig. 5). A duct leads about halfway into the gland. Distal cells of the gland radiate from the duct and are largely vacuolated, with small, rounded, deeply staining bodies, probably nuclei (Figs. 6-7). At the base of the gland often six to eight vitelline follicles are closely clustered together and more or less enclosed by the fibrous lining of the gland (Fig. 6). This lining seems to be derived from the fibrous stroma of the parenchyma but it becomes less distinct near the base of the gland. Between these vitelline follicles and the distal cells occur a few, large, thin-walled cells which appear like disintegrating vitelline cells. They are filled with finely granular, yellowish, non-staining particles. However, some ventral glands are well developed before the vitelline follicles are differentiated and even in mature specimens some glands show no connections with vitellaria; for example, the two glands at the anterior border of the acetabulum. The glands directly opposite the three gonads are among the first to develop and usually bulge out, hernia-like, from the ventral surface. In some specimens much of the testes and almost the entire ovary and uterus seem to have moved into these sac-like bulges of the ventral surface of the body. A similar invasion of ventral glands by the testes, ovary, vitellaria, and uterus has been noted in the case of a monostome, *Quinqueserialis quinqueserialis* (Barker and Laughlin, 1911) (= *Notocotyle quinqueserialis*), by Barker and Laughlin (1911).

The presence of such ventral glands is unusual among trematodes and, so far as I can determine, previously undescribed for distomes. Papilla-like and retractile glands are well known in the monostome family NOTOCOTYLIDAE. Although the NOTOCOTYLIDAE are very unlike *Dermadena* in lacking an acetabulum, a pharynx, and a seminal receptacle, there are some interesting similarities in addition to the rows of ventral glands. The body of notocotylids is often concave ventrally and scaled or spined; the excretory pore is dorsal and far forward; the Y-shaped excretory vesicle of notocotylids is at least simulated in *Dermadena*; in both, the testes are symmetrical; the ovary is intertesticular; the cirrus sac and metraterm are both well developed; an external seminal vesicle is present. In addition, notocotylids sometimes possess the short diverticula of the ceca and a tuberculated cirrus. Since there is no doubt regarding the relationship of *Dermadena* to *Pseudocreadium*, a genus commonly recognized as in the family LEPOCREADIIDAE, evidence points to relationship between the monostome family NOTOCOTYLIDAE and the distome family LEPOCREADIIDAE. The few life cycles known in these two families include development of oculate cercariae in rediae, although the cercaria of *Notocotylus* is a monostome cercaria and the cercaria of *Lepocreadium* is distome and trichocercous. No life cycle of the genus *Pseudocreadium* is known.

*Pseudocreadium lamelliforme* (Linton, 1907), n. comb.

In 1907, Linton named *Distomum lamelliforme* from *Balistes capriscus* Gmelin (= *Balistes carolinensis* Gmelin), the triggerfish, and from *Lactophrys tricornis* (Linn.) and *L. trigonus* (Linn.) trunkfishes, at Bermuda. It is clear from Linton's description and figures and from study of type and other specimens from *Balistes*, the type host, that *Distomum lamelliforme* belongs in the genus *Pseudocreadium*, and that the specimens from trunkfishes are *Dermadena lactophrysi*. Linton did not describe or figure the ventral glands of the latter but the arrangement and size of

the structures he termed vitellaria in the specimens from trunkfishes make it clear that these were actually groups of vitellaria at the bases of the glands. The following measurements of *P. lamelliforme* are from three specimens on the slide of type specimens and six specimens from the "turbot" (*Balistes*, probably *B. capriscus*) at Bermuda. Length 0.999 to 1.952 mm, width 0.931 to 2.133 mm; oral sucker 0.082 to 0.142 mm in diameter; acetabulum 0.122 to 0.210 mm in diameter; sucker ratio 1:1.23 to 1.62; eggs 63 to 68 by 34 to 44  $\mu$ .

*P. lamelliforme* is so similar to *P. scaphosomum* Manter, 1940, that they should perhaps be considered the same. Sizes and sucker ratios agree. Eggs of *P. lamelliforme* average larger, with both upper and lower limits larger, although there is overlapping. The only detail observed which seemed to be constantly different is the shape of the external seminal vesicle. In all 47 specimens of *P. lamelliforme*, the external seminal vesicle is straight and sac-like, whereas in all of 31 specimens of *S. scaphosomum* the vesicle is tubular and sinuous, curved at least once and commonly S-shaped. Like *P. scaphosomum*, *P. lamelliforme* has a bipartite prostatic vesicle and when retracted the cirrus is somewhat folded or curved within the sac. *P. balistes* Nagaty, 1942, seems to differ chiefly in its bipartite external seminal vesicle and somewhat larger eggs.

#### SPECIES OF *Pseudocreadium*

The following eight species have been named in the genus *Pseudocreadium*: *P. monocanthi* Layman, 1930 (synonym: *Leptocreadium skrjabini* Ozaki, 1936—pointed out by Yamaguti, 1938); *P. vitellosum* (Ozaki, 1936) Yamaguti, 1938; *P. symmetrorchis* (Ozaki, 1936) Manter, 1940; *P. patellare* (Yamaguti, 1938) Manter, 1940; *P. scaphosomum* Manter, 1940; *P. spinosum* Manter, 1940; *P. sohali* Nagaty, 1942; *P. balistes* Nagaty, 1942; *P. elongatum* Nagaty, 1942; *P. lamelliforme* (Linton, 1907). Certain of these species, notably *P. elongatum* and *P. vitellosum*, are so similar to *Lepocreadium* that generic distinction between *Lepocreadium* and *Pseudocreadium* becomes questionable. Although the type species of *Lepocreadium*, *L. album*, has tandem testes and unlobed ovary, the genus, probably correctly, now includes species with diagonal testes and with lobed ovary, and some with ovoid as well as elongated bodies. Probably the best character to separate the genera is the symmetrical position of the testes in *Pseudocreadium*. In the type species, *P. monocanthi*, the multilobed ovary is directly anterior to one testis, in all other species with symmetrical testes the ovary is more or less intertesticular. It is suggested that species with diagonal testes be transferred to *Lepocreadium*, becoming *Lepocreadium vitellosum* (Ozaki, 1936), n. comb.; *L. sohali* (Nagaty, 1942), n. comb.; and *L. elongatum* (Nagaty, 1942), n. comb. Most species of *Lepocreadium* possess a terminal or subterminal excretory pore, while in *Pseudocreadium* the pore is dorsal, conspicuous, and relatively far forward. *L. vitellosum*, however, has the *Pseudocreadium* type of pore. The two genera approach each other so closely that separation must, at present, be more or less arbitrary.

Manter (1940) considered *Hypocreadium* Ozaki, 1936, a synonym of *Pseudocreadium*, pointing out individual variability of spination, lobation of ovary, and posterior extent of uterus. This view is still held, although a possible distinction could be the preovarian seminal receptacle and the intertesticular ovary in *Hypocreadium*.

The excretory vesicle in *Pseudocreadium*, as in *Dermadena*, usually appears to be Y-shaped due to the inflation of the lateral tubes. Sections of *P. scaphosomum* show that, in that species at least, the lateral vessels open into the vesicle slightly posterior to its anterior end.

*Pseudocreadium galapagoensis* n. sp.

In a restudy of the confusing individual variation of specimens of *P. scaphosomum*, it was discovered that the author's collection from *Balistes verres* from the Galapagos Islands contained two species rather than one. Of 13 specimens, 9 are small-sized *P. scaphosomum* as reported; the other 4 specimens are a new species. Five specimens collected from the same host, *B. verres*, from Isabel Island, Mexico, are all *P. scaphosomum*.

*Pseudocreadium galapagoensis* n. sp.

(Fig. 9)

*Description* (based on 4 specimens): Body slightly longer than wide, edge slightly inrolled ventrally in posterior half; 0.825 to 1.107 mm long by 0.750 to 0.931 mm wide; smooth except for a few fine scales on edges of suckers; oral sucker 0.127 to 0.217 mm in transverse diameter; acetabulum 0.144 to 0.225 mm; sucker ratio 1:1 to 1.13. Prepharynx short; pharynx relatively large, 0.102 to 0.136 mm long by 0.090 to 0.136 mm wide; esophagus short; ceca bow anteriorly and outward, then extend almost straight back until they curve medianly posterior to the testes, with short lateral and median bulges in posterior half. Genital pore to the left, opposite or just median to left cecum, about halfway between suckers. Testes symmetrical, lobed, in posterior half of body. Cirrus sac extending from genital pore almost directly backward along left margin of acetabulum to midacetabular level or slightly beyond; containing a spherical seminal vesicle, prostatic vesicle with wide posterior and narrow, tubular, sinuous anterior, portions; and sinuous cirrus. External seminal vesicle small, tubular, sinuous. Ovary of irregular, variable shape; seminal receptacle large, elongated, directly to left of ovary or to the left and slightly anterior, not overlapping acetabulum; uterus preovarian, extending along the right side of the acetabulum to or slightly beyond anterior edge of the acetabulum, then across the body posterior to the acetabulum to join the metraterm to the left of the cirrus sac; metraterm as long as or slightly longer than the cirrus sac, its posterior tip narrowed, appendix-like. Vitelline follicles filling sides of body from oral sucker to posterior end, confluent dorsally between pharynx and acetabulum and posterior to testes, continuous across ceca dorsally but not ventrally. Eggs 60 to 65 by 33 to 34  $\mu$ . Excretory pore dorsal at level of posterior ends of testes; excretory vesicle traceable only to the ovary.

*Host*: *Balistes verres* (Gilbert and Starks).

*Location*: Intestine.

*Locality*: Charles Island, Galapagos.

*Type specimen*: U. S. Nat. Mus. Helminth. Coll., No. 36932.

*Comparisons*.—The most characteristic feature of this species is the fact that both cirrus sac and metraterm lie parallel and close together on the left side of the acetabulum. In all other species these two organs diverge from the genital pore toward opposite sides of the acetabulum. The anterior coil of the uterus on the right side of the acetabulum and the postacetabular seminal receptacle are other peculiar characters. The suckers are more nearly the same size and the pharynx is relatively larger than in other species. *P. galapagoensis* is like *P. spinosum* in the preacetabular continuity of the vitellaria.

SUMMARY

1. A new genus, *Dermadena*, with type species *D. lactophrysi*, is named from trunkfishes at Tortugas, Florida.

2. The presence in *Dermadena* of rows of conspicuous ventral glands suggests similar glands known in the monostome family NOTOCOTYLIDAE and a possible relationship of the LEPOCREADIDAE and the NOTOCOTYLIDAE.

3. *Distomum lamelliforme* Linton, 1907, is referred to the genus *Pseudocreadium*.
4. *Pseudocreadium vitellousum* Ozaki, 1936; *P. sohali* Nagaty, 1942; and *P. elongatum* Nagaty, 1942, are considered to belong in the genus *Lepocreadium*.
5. *Pseudocreadium galapagoensis* n. sp. is named for four specimens occurring with *P. scaphosomum* Manter, 1940, in *Balistes verres* from the Galapagos Islands.

## REFERENCES

- BARKER, FRANKLIN D. AND LAUGHLIN, JOSEPH W. 1911 A new species of trematode from the muskrat, *Fiber zibethicus*. Trans. Am. Micr. Soc. 30: 261-274.
- LAYMAN, E. M. 1930 Parasitic worms from the fishes of Peter the Great Bay. Bull. Pacific Sc. Fishery Res. Sta. 3(6): 1-120. (Russian, with German summary.)
- LINTON, EDWIN 1907 Notes on parasites of Bermuda fishes. Proc. U. S. Nat. Mus. 33: 85-126.
- MANTER, HAROLD W. 1940 Digenetic trematodes of fishes from the Galapagos Islands and the neighboring Pacific. Allan Hancock Pacific Exped. 2(14): 329-497.
- NAGATY, H. F. 1942 Trematodes of fishes from the Red Sea. Part 3—On seven new allocreadiid species. Fouad I. Univ., Pub. Mar. Biol. Sta. Ghardaoua (Red Sea), No. 4: 1-27.
- OZAKI, Y. 1936 Two new genera of the trematode family Allocreadiidae. Zool. Mag. 48: 513-519. (Japanese, with English summary.)
- YAMAGUTI, SATYU 1938 Studies on the helminth fauna of Japan. Part 21. Trematodes of fishes, IV. 139 pp. Privately published: Maruzen Co., Tokyo.

## EXPLANATION OF PLATE

All figures were made with the aid of a camera lucida. The projected scales have the values indicated in mm.

## ABBREVIATIONS

cir	cirrus	gp	genital pore
cs	cirrus sac	mt	metraterm
ep	excretory pore	ov	ovary
esv	external seminal vesicle	prv	prostatic vesicle
ex	excretory vesicle	sr	seminal receptacle
gl	ventral gland		



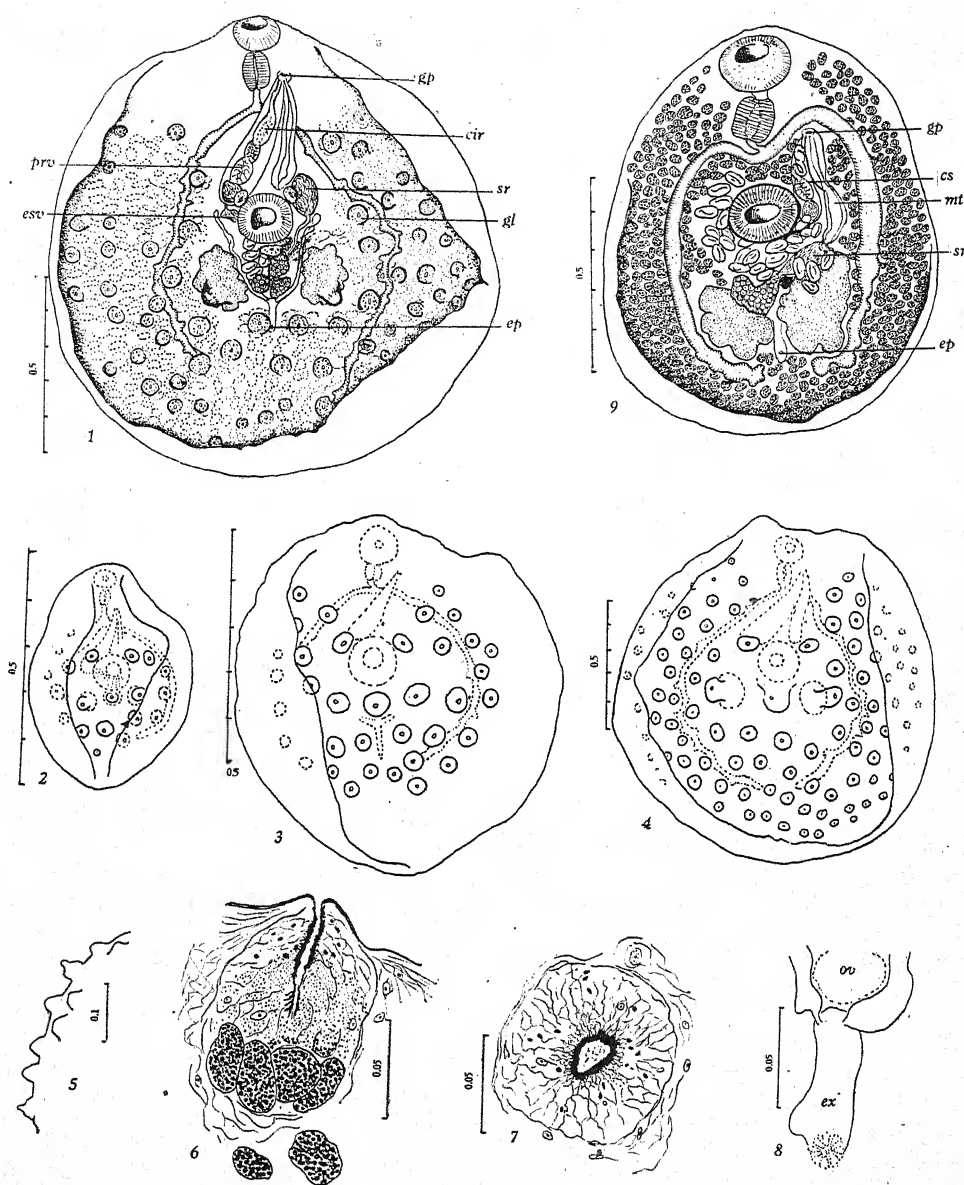


FIG. 1. *Dermadena lactophrysi*. Ventral view.

FIGS. 2-4. *D. lactophrysi*. Three specimens, 0.5, 0.75 and 1.376 mm in length, drawn to show number and position of ventral glands. Note change in scale in Fig. 4.

FIG. 5. Outline of portion of ventral body wall of *D. lactophrysi* showing protruded glands.

FIG. 6. Longitudinal section of a ventral gland of *D. lactophrysi* showing duct, vitelline follicles, and distal cells.

FIG. 7. Cross section through distal portion of a ventral gland of *D. lactophrysi*.

FIG. 8. Excretory vesicle of *D. lactophrysi*.

FIG. 9. *Pseudocreadium galapagoensis*. Ventral view.

REDESCRIPTION OF THE SPECIES OF *GYROCOTYLE* FROM THE  
RATFISH, *HYDROLAGUS COLLIEI* (LAY AND BENNET), WITH  
NOTES ON THE MORPHOLOGY AND TAXONOMY  
OF THE GENUS

JAMES E. LYNCH

School of Fisheries, University of Washington, Seattle, Washington

I. INTRODUCTION

In 1911 Edna E. Watson described *Gyrocotyle fimbriata* n. sp. from the ratfish, *Hydrolagus colliei*, collected on the California coast, and also reported the presence of a second species from the same host which she designated "*G. urna* (var.?)." Unfortunately Watson did not publish a detailed description of the second species, which she considered to be a variety of, if not actually identical with, the *G. urna* from *Chimaera monstrosa*.

Ward (1912) published a study of *Gyrocotyle* from the same host, but implied that there is only one species and that Watson had designated as separate species different stages in the contraction of the worm, with consequent differences in the amplitude and complexity of the lateral plications and of the folds of the rosette. Apparently, Ward considered *fimbriata* to be an invalid species, although he did not clearly say so.

Dollfus (1923) states that Ward was right in concluding that the two forms described by Watson belong to a single species (p. 236-237) and that *G. fimbriata* Watson is a synonym of *G. urna* Grube & Wagener (p. 238).

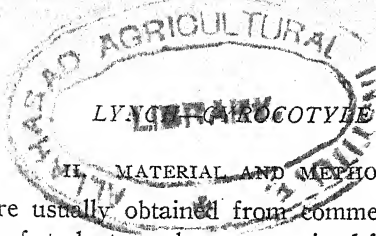
Wardle (1932) states that the *Gyrocotyle* from *Hydrolagus colliei* belongs to one species, *G. urna*, identical with the species from the Atlantic *Chimaera monstrosa*.

None of these authors, in disputing the presence of two species of *Gyrocotyle* in the ratfish, took into consideration any of the valid specific differences between them other than the differences in the complexity and amplitude of the plications of the lateral borders and of the rosette, which they regarded as inconstant and varying with the contortions of the worm. Ward and Wardle, at least, had both species at their disposal; it seems likely that Dollfus examined specimens of *G. fimbriata*, only.

In the past decade I have collected and examined hundreds of specimens of *Gyrocotyle* from ratfishes taken in Puget Sound and along the coast of Washington. The two forms described by Watson definitely exist, and there is no reason for not regarding them as distinct species. It is the purpose of this paper to describe both species with sufficient completeness for their subsequent recognition, and also to point out the inadequacies in the descriptions of several other species and alleged species of the genus in the hope that their precise taxonomic status will be defined by zoologists who have ready access to the hosts. Finally, certain disputed points in the interpretation of the morphology and development of the genus *Gyrocotyle* will be considered.

For details of the histology and cytology of the genus the reader is referred to the publications of Lönnberg (1891) and Watson (1911).

Received for publication, September 21, 1945.



## II. MATERIAL AND METHODS

The ratfishes were usually obtained from commercial fishermen or from the collecting expeditions of students, and were examined from four to forty-eight hours after capture. Usually, at least twenty-four hours had elapsed from the time the fish were taken until the parasites could be removed. The intestine of the fish was removed and slit open in a dish of 0.7 per cent saline solution. The *Gryocotyle* were usually alive and in good condition, although the intestinal mucosa had sloughed off in the majority of the hosts at the time of examination. The worms were kept in the saline solution, or more rarely in sea water, until preservation. *Gyrocotyle* is notoriously difficult to fix in an extended and undistorted condition. The most satisfactory procedure was to narcotize them in the saline solution with menthol-chloral hydrate (Galigher, 1934). By the end of from six to eighteen hours some of the worms would be more or less normally relaxed, others would remain badly contracted. Relaxed specimens were dropped into 8% formalin in normal saline solution, or were fixed in saturated sublimate solution with 5 per cent acetic acid. Specimens which showed a tendency to curl were placed on a glass plate, straightened with camel's hair brushes, and flooded with the fixative. A few were fixed while strongly compressed between glass plates, but the resultant distortion nullified any advantages in the thinner specimens so obtained. Practically all stains adapted for whole mounts were tested on *Gyrocotyle*. Best results were obtained by staining with Galigher's (1934) modification of Harris's haematoxylin diluted with 9 parts of a saturated solution of ammonia alum. Carmines and cochineals have the disadvantage of leaving the spines unstained and practically invisible, and of not producing enough contrast between internal organs and the surrounding parenchyma. Alum haematoxylin usually stains the body spines, and sometimes the acetabular spines as well, and in addition produces more contrast between the organs of the reproductive system and the surrounding tissues.

## III. ACKNOWLEDGMENTS

The writer is especially indebted to Dr. Kelshaw Bonham, Mr. Frederick C. Cleaver and Mr. R. T. Smith of the State Department of Fisheries, to Captain Walt Hossfeld of the trawler "St. John" and to Captain Edwin Smith of the trawler "Garnet C." for donations of large numbers of ratfishes. Various other friends and students, too numerous to mention, have contributed occasional specimens. The photographs of Plate III were taken by Dr. Bonham.

## IV. DEFINITION OF TERMS USED

In the descriptions which follow, the end with the acetabulum is considered anterior, the end with the funnel and rosette is considered posterior. The side bearing the small proximal pore of the funnel and the vaginal pore is designated dorsal, the side bearing the uterine pore and the male genital pore is ventral. Mature specimens are those bearing eggs in the uterus; immature specimens are those with adult characters, but without eggs in the uterus. Postlarval (juvenile) forms are those with reproductive organs incompletely developed and with incompletely formed plications in the rosette.

The funnel is the conical cavity extending from the posterior end of the body to the dorsal pore. The rosette is the elaborately plicated distal (posterior) border



of the funnel. The cephalic cone (Ward, 1912) is the region anterior to the genital notch and genital pores.

For convenience in description, the cuticular spines are considered to occur in the following groups: (1) acetabular spines, comprising two groups of large spines near the anterior end, to the right and left of the acetabulum; (2) antero-lateral spines, forming a file of small spines along the lateral border between the acetabular group and the level of the anterior end of the uterine sac; (3) body spines, on the dorsal and ventral surfaces of the body. They are largest posteriorly and diminish in size gradually from behind anteriorly; (4) marginal spines, along the right and left margins of the body, posterior to the anterolateral spines. The last three categories of spines may not be sharply separated one from the others.

Unless otherwise stated, all data and discussions of a taxonomic character refer to mature specimens in a state of normal extension. Highly contracted, contorted or immature specimens were not used for measurements or comparisons.

With the partial exception of the inconspicuous and rather variable spines, *Gyrocotyle* has no structure of constant dimensions or pattern which can be used for specific identification. Since the animal is not only highly contractile and extensile, but also a gifted contortionist, and no two preserved specimens have precisely the same proportions or appearance, comparisons between species must be made to a large extent on the basis of the relative proportions, or degree of development, of conspicuous external and internal morphological features. Such a method is practicable in this case. If specimens of the two species of the same approximate size and state of extension are compared, homologous organs of the two species always show the same relative differences in size and position. Some structures of the two species present an absolute difference. For example, the length of the testicular areas in proportion to the length of the body, or the posterior limit of the vitelline glands, are constant differences without specific intergradations.

Several conspicuous morphological features distinguishing the two species are not amenable to convincing description. Such is the case with the size, number and amplitude of the undulations of the lateral borders of the body, and with the relative complexity of the rosette. Actually, the two species can be distinguished, almost invariably, on the basis of these two features alone. Contrary to the implications of several contributors to the literature on *Gyrocotyle* (Ward, 1912; Fuhrmann, 1930-31; Wardle, 1932), contraction or elongation does not convert the morphology of one into that of the other species. The species which, when elongated, has few and simple lateral undulations and a small, simple rosette, still has these features when contracted if compared with the other more complex species in the same stage of contraction.

#### V. TAXONOMY AND MORPHOLOGY

Genus *Gyrocotyle* Diesing, 1850 (Cestoda: Cestodaria)

*Synonymy:* *Amphiptyches* Grube and Wagener 1852; *Crobylophorus* Krøyer 1852-53.

*Genotype:* *G. rugosa* Diesing, 1850.

*Diagnosis:* Body of one segment, dorsoventrally flattened, in average extension about three and three-fourths times longer than wide and one-fifth to one-fourth as thick as broad, but highly extensile and contractile. At the anterior end a powerful muscular sucker (acetabulum); at the posterior end a funnel-shaped haptor with a small anterior dorsal pore and a wide posterior opening, the thin borders of which are more or less complexly folded (the rosette). Lateral borders of the body thinner than the median portion, simple, undulant or complexly ruffled. Cuticular spines present. Excretory pores paired, dorsal, in the anterior fourth of the body,



near the junction of the thicker median and thinner lateral regions. Vaginal pore dorsal, to right of the median line, in the anterior fourth of the worm. Ovary, shell gland and seminal receptacle in the third quarter of the body. Uterus, courses anteriorly in spiral windings to open into a large saccate uterine pouch, the pore of which is ventral and median, in the anterior fourth of the body. Vitelline follicles extend almost the length of the body, are most numerous laterally and largely absent in the median reproductive area. Testicular follicles numerous, in right and left groups, in the anterior half of the body. Vasa efferentia empty into a coiled vas deferens which discharges into the ejaculatory duct through a valvelike papilla. Male genital pore ventral, to the right of the median line, in the anterior fourth of the body. Eggs thin-shelled, operculate. Larva ciliated, free-swimming, with a protrusible papilla bearing ten hooks at its posterior end. Life history incompletely known. Adults in spiral valve of Chimaerid fishes.

*Gyrocotyle fimbriata* Watson, 1911

(Fig. 1)

(For synonymy see Section X: Valid species of *Gyrocotyle*.)

**Diagnosis:** With the characters of the genus. Mature specimens 32 (13–63) mm in length. Acetabulum 1.86 (1.5–2.55) mm long; 0.90 (0.65–1.4) mm wide; slightly compressed dorsoventrally. Rosette large, its diameter 82 (60–112) per cent of greatest width of body, intricately folded, usually in contact with the posterior ruffles of the lateral margins. Undulant lateral borders extend from near the level of the genital pores to a short distance posterior to the dorsal pore of funnel, and are thrown into numerous, 31 (18–60), highly developed ruffles, the dorsoventral amplitude of which exceeds the maximum thickness of the body, and which are often replicate and secondarily undulant. Acetabular spines 25 to 50 in each group, not sharply set off from the anterolateral spines, the largest 175 (84–300)  $\mu$  in length, from 6 to 13 per cent of the length of the acetabulum. Body spines encircle the posterior half of the funnel region anterior to the rosette, whence they extend forward dorsally to the level of the posterior part of the ovary, or very rarely to the posterior end of the uterine sac. Ventrally, the body spines rarely extend anteriorly to the funnel region. Posterior body spines 148 (75–250)  $\mu$  long, sometimes larger than the acetabular spines. Scattered small spines occur along the margins of the lateral undulations in some specimens.

Excretory pores inconspicuous, dorsal, usually posterior to the level of the anterior end of the uterine sac, 3.4 (2.6–4.7) times the length of acetabulum from the anterior end. Testicular follicles in right and left groups, extending from near the middle of the acetabulum to, or beyond, the posterior end of the uterine sac. Left testicular area occupies 33 (24–45) per cent of total length of the worm. Vas deferens large, convoluted, conspicuous, discharging into the ejaculatory duct by a valve-like papilla surrounded by a conspicuous muscular bulb, both developed from the proximal end of the ejaculatory duct. Male genital pore ventral, slightly to right of median line, 11.5 (8.3–14.8) per cent of total length of the worm from the anterior end. Vaginal pore dorsal, about three-fourths of the distance from the median line to the lateral margin, and 10.7 (7.5–15) per cent of body length from the anterior end. Follicles of vitelline glands extend from about the level of the middle of the acetabulum to the level of the anterior border of the rosette.

Eggs spontaneously discharged from living worms 96.5 (90–107)  $\mu \times$  72 (58–78)  $\mu$ , surrounded by a transparent jelly 5.5–9  $\mu$  thick; operculum present, with nearly invisible suture. Egg contents: an embryo in early stage of development, surrounded by vitelline cells.

**Hosts:** *Hydrolagus colliei* (Lay and Bennett) and *Chimaera monstrosa* L.

*Gyrocotyle urna* (Grube and Wagener, 1852)

(Fig. 2)

**Synonymy:** *Amphiptyches urna* Grube u. Wagener (in Wagener, 1852); *Gyrocotyle amphiptyches* Wagener, 1858; *Gyrocotyle nigrosetosa* Haswell, 1902.

(Following description based on specimens from *Hydrolagus colliei*.)

**Diagnosis:** With the characters of the genus. Mature specimens 36 (14–55.5) mm in length. Acetabulum 2.6 (1.7–2.75) mm long; 1.1 (0.93–1.45) mm wide, slightly compressed dorsoventrally. Diameter of rosette 45 (35–60) per cent of greatest body width, with few and simple plications. Thinner lateral margins of body with few, 15 (8–30), undulations which extend posteriorly from near the level of the genital pores to disappear between the level of the ovary and dorsal pore of the funnel; the undulations may be absent in small specimens. Acetabular spines compactly grouped, 17 to 25 on each side, the largest 200–800  $\mu$  long; from 9 to 27 per cent of the length of the acetabulum. Body spines numerous, extending from mid-region of funnel anteriorly to the region of the uterine sac on the dorsal side; ventrally, they extend anteriorly little beyond the level of the dorsal pore. Marginal spines may occur the entire length of the worm, but usually are large, numerous and conspicuous only posterior to the level of the ovary. Posterior dorsal spines large (150–360  $\mu$ ) becoming gradually smaller anteriorly.

Excretory pores conspicuous, dorsal, usually anterior to the level of anterior end of uterine sac, 1.7 (1.3–2.5) times the length of acetabulum from the anterior end. Testicular follicles in right and left groups, extending from about the level of the posterior fourth of acetabulum to the level of the anterior border of the uterine sac, or rarely to the middle thereof. Left testicular field 11 (7–15) per cent of total length of worm. Vas deferens inconspicuous, feebly convoluted. Ejaculatory duct with papilla in its proximal end which is not surrounded by a conspicuous muscular bulb. Male sex pore ventral, slightly to the right of median line, 10.6 (7.8–14.7) per cent of total length of worm from the anterior end. Vaginal pore dorsal, usually about half way between the median line and lateral border, 9.4 (7–14.1) per cent of total body length from anterior end. Vitelline glands extend from the middle of acetabulum to a level anterior to the dorsal pore of funnel.

Spontaneously deposited eggs 94 (81–111)  $\mu$  by 65.5 (55–72)  $\mu$ , operculate, but with nearly invisible suture; entire egg surrounded by a transparent jelly 6–12  $\mu$  thick. At time of deposition, egg contains an embryo in early stage of development surrounded by vitelline cells.

*Two varieties:* *Forma magnispinosa*, with largest acetabular spines 420–800  $\mu$  long, always longer than body spines. *Forma parvispinosa*, with largest acetabular spines 220–360  $\mu$  long, usually smaller than largest body spines.

*Hosts:* *Hydrolagus collei* (Lay and Bennet); *Chimaera monstrosa* L.; *Chimaera olgibyi* Waite; and doubtfully *Callorhynchus antarcticus* Lacépède.

#### Discussion of Specific Differences

1. *External appearance.*—The two species can usually be distinguished with the unaided eye by the following criteria: *G. fimbriata*, as compared with *G. urna*, is more robust, the lateral ruffles are more numerous and protruding, the rosette much larger and more intricate, the sides of the body more nearly parallel with less taper in the posterior fourth, the “cephalic cone” is slightly larger and more conspicuous, and the body spines with their papillae are not evident.

2. *Size and complexity of the rosette.*—In all specimens past the postlarval stage the rosette (the expanded, plicate border of the funnel) is enormously more complexly folded and crispate in *G. fimbriata* than in *G. urna*. In addition it is relatively nearly twice as wide, on the average, as in *G. urna*, although the mobility of the worms results in an occasional specimen of *fimbriata* presenting a contracted rosette of *urna*-like dimensions, but not of *urna*-like simplicity.

3. *The lateral ruffles.*—In *G. fimbriata* the lateral undulations are usually more numerous than in *G. urna*, always of greater dorsoventral amplitude, always more outstanding and distinctly separated from the median thickened part of the body, usually more flaring at their outer borders, and are more likely to possess secondary undulations or lobes. If one semicircular curve of the border be considered a ruffle, *G. fimbriata* will possess, on the average, twice as many ruffles as *G. urna*.

4. *The cuticular spines.*—In living specimens of both species the spines of all parts of the animal are usually colorless and glassy, although occasionally a light yellow. They are rather soft, and shrink more or less obviously as the result of the procedures involved in making microscopic preparations. The spines are composed of concentric layers of cuticular substance. Down the axis is a column of non-laminated material, apparently the pulpy remains of the earliest layers. The laminated nature of the spines is easily seen in live material, or in preserved specimens cleared in glycerine. In balsam mounts the lamination is less distinct and often the superficial layer stains much more deeply than the interior layers.

Rare specimens of both species have been encountered with black spines. These specimens always came from dead ratfishes in which the intestinal contents were blackened and putrid. The spines evidently had absorbed the black color from the decomposing bile and food residue, since the projecting tips were much blacker than

embedded parts, and the outer layers darker than the more axial portions. Black spines cannot be considered a specific character (Haswell, 1902), and the acetabular spines of *G. fimbriata* are not typically black as stated by Watson (1911, p. 369).

(a) *Acetabular spines*.—The acetabular spines are difficult to count accurately in whole mounts. In *G. fimbriata* they rarely stain. In *G. urna* they sometimes stain distinctly with alum haematoxylin. They are arranged in imperfectly longitudinal and obliquely dorsoventral rows; thus present an imperfectly quincuncial arrangement in surface (lateral) view. In the few specimens examined in this way there were from 5 to 8 longitudinal rows in *G. fimbriata* and 4 or 5 in *G. urna*.

In *G. fimbriata* the acetabular spines (Figs. 19–23) are small, 175 (84–300)  $\mu$  long by 31 (7–65)  $\mu$  in maximum diameter. The shape is stubby, much like a tenpin, but often asymmetrical or clublike.

In *G. urna magnispinosa* the acetabular spines (Figs. 9–10) are extremely large, 578 (420–800)  $\mu$  long by 54 (40–85)  $\mu$  in maximum diameter. The shape is graceful and symmetrical. In *G. urna parvispinosa* (Figs. 14–17) the size, 250 (180–360)  $\mu$  by 34 (25–55)  $\mu$ , averages larger than in *G. fimbriata*, although the size ranges overlap. In shape they resemble those of *f. magnispinosa*, although less elegant and symmetrical.

There is no evidence that *f. parvispinosa* is a stage in the development of *f. magnispinosa*. It is possible, however, that a study of a large enough series of *G. urna* might reveal intergradations between the two presumptive varieties.

(b) *The body spines*.—At the posterior end of *G. fimbriata* the body spines (Figs. 24–29) encircle the funnel region, and are found quite to the base of the plications of the rosette. In a third of the specimens they are set in inconspicuous papillae. On the dorsal side they extend forward about as far as the pore of the funnel medially, but laterally may reach about as far as the level of the posterior boundary of the ovary or seminal receptacle. *Rarely*, they extend forward, diminishing in size and becoming more widely separated, to the level of the posterior part of the uterine sac. On the ventral side they rarely extend forward more than three-fourths of the way from the rosette to the level of the funnel pore, and never further anteriorly than the ovary. The average size is  $148 \times 37 \mu$ , but they may vary from  $35 \times 12 \mu$  to  $250 \times 50 \mu$ . Usually they are smaller than the acetabular spines, but in 20 per cent of the specimens are somewhat larger.

In some individuals they are so minute and scattering that the worm is practically spineless. It seems possible that very few individuals of *G. fimbriata* are naturally devoid of body spines. Juvenile specimens, or even small mature ones, often have a row of closely set spines extending the entire length of the lateral margins. In mature adults these are usually reduced to a few anterolaterals and a few on the ruffles of funnel region, true marginal spines along the borders of the ruffles being very sparse or even apparently absent altogether.

In *G. urna* the body spines (Figs. 11–13, 18) encircle the body in the anterior half of the funnel region, thus do not occur as far posteriorly as in *G. fimbriata*. On the dorsal side they extend anteriorly, usually embedded in distinct elevated papillae, for 80 per cent of the length of the worm. In the median area, overlying the principal reproductive organs, they extend forward as far as the anterior end of the spiral uterus, or the posterior fourth of the uterine sac. Laterally, they extend forward for one-half to three-fourths the length of the uterine sac and may even



extend to, and merge with, the anterolateral spines. The dorsal spines occur in irregular and undulating transverse rugae, each spine on a distinct papilla. The arrangement is imperfectly quincuncial. Contrary to the statement of Watson (1911, p. 370) the "pattern" is much more distinct in *urna* than in *fimbriata*. The body spines are largest and most closely set in the posterior part of the body; anterior to the level of the ovary they become more and more widely spaced and gradually smaller.

The ventral body spines range from the middle of the funnel region to the level of, or slightly anterior to, the dorsal pore of the funnel. Laterally, they merge with the marginal spines. Often the ventral spines are so sparse as to form no distinct group.

The largest body spines, on the posterior dorsal or lateral surfaces, are about the same size in the varieties with the large and small acetabular spines;  $235 (150-360) \mu \times 50 (22-90) \mu$  in the former and  $262 (190-325) \mu \times 49 (35-75) \mu$  in the latter. In *f. magnispinosa* they are never as large as the acetabular spines; in *f. parvispinosa* they are slightly larger than the acetabular spines in about one out of five individuals.

In *G. urna* the marginal spines may be large, conspicuous and numerous the entire length of the lateral margins, or few or absent in the anterior one-half to two-thirds of the margins, although always abundant from the level of the ovary backward.

5. *The excretory pores (ex.p., Figs. 1-2).*—The excretory pores are located dorsally near the junction of the thicker median and thinner lateral portions of the body, a short distance posterior to the level of the vaginal pore. In *G. fimbriata* the excretory pores are extremely inconspicuous. However, they can be located in most stained whole mounts by one familiar with their appearance and location. They are usually posterior to the level of the anterior border of the uterine sac. In *G. urna* the excretory pores are much more conspicuous, and often have a distinct swollen vesicle just beneath the pore. They are usually anterior to the level of the anterior end of the uterine sac, distinctly more anterior than in *G. fimbriata*.

6. *The uterine pore.*—The uterine pore (*ut.p.*, Fig. 2) is located on the ventral surface, in the median line, a short distance back of the anterior end of the uterine sac. It is patent and readily seen only in a minority of specimens. In percentages of the total length of the worm, the uterine pore is 18% (12-24) per cent distant from the anterior end in *G. fimbriata* and 15 (12-23) per cent in *G. urna*.

7. *Testes.*—The testes occur as a large number of spheroidal follicles arranged in two fields, right and left, in the medulla of the anterior end of the body. The fields are separated by the uterine sac, vas deferens, ejaculatory duct and part of the vagina. In about half of the specimens of both species a few testicular follicles bridge over the gap between the two fields just behind the acetabulum. The right testicular field is divided into anterior and posterior portions by the vagina which crosses it.

In *G. fimbriata* the testicular follicles are very numerous, crowded, and form deeply staining compact groups extending from the middle of the acetabulum to, or beyond, the posterior end of the uterine sac in 88 per cent of the cases, and posterior to the middle of the uterine sac in all cases.

In *G. urna* the follicles are much fewer in number, often more widely spaced, and



of smaller average size. The testicular fields extend from the posterior fourth of the acetabulum to the anterior part of the uterine sac. In 37 per cent of the specimens they reach only to the anterior border of the uterine sac; in 45 per cent they extend only one-third the length, and in 18 per cent one-half the length of the uterine sac. The compact parts of the testicular fields are relatively shorter than in *G. fimbriata*, without exception, although in rare specimens a few scattered follicles follow the course of the nerve cords for a short distance further.

8. *The male pore.*—The pore of the ejaculatory duct is located ventrally, slightly to the right of the median line. In terms of total body length, its average distance from the anterior end is 10.8 per cent in *G. urna* and 13.3 per cent in *G. fimbriata*. However, the pore is relatively much farther from the anterior end in small specimens (13.7 per cent in *G. urna*; 16.7 per cent in *G. fimbriata*) and closer in those of maximum size (8.4 per cent in *G. urna*; 9.2 per cent in *G. fimbriata*).

In both species the male pore is usually slightly posterior to the level of the vaginal pore, rarely at the same level or slightly anterior thereto.

9. *The vas deferens and ejaculatory duct* (Figs. 38–39).—As seen in whole mounts, the true length of the ejaculatory duct cannot be measured, since it slopes ventrally from its posterior to its anterior end and also curves downward and to the right. It is usually foreshortened by about 20 per cent, as seen in dorsal or ventral view. The measurements used in the following comparison are its anteroposterior extent, and not its actual length. The ejaculatory duct (and also the anterior, thickened part of the vagina) seems to be independently contractile and extensile, which probably accounts for the rather great range in its length.

In *G. fimbriata* the ejaculatory duct has an anteroposterior extent of 1.6 (1.1–2.7) mm, which is 98 (65–150) per cent of the length of the adjacent thickened part of the vagina. In *G. urna* the anteroposterior extent of the ejaculatory duct is 0.9 (0.6–1.15) mm; only 48 (32–74) per cent as long as the thickened part of the vagina. Consequently, the ejaculatory duct of *fimbriata* is nearly twice as long as that of *urna* on the average, and is also longer relative to the thickened part of the vagina.

At the fundus, or posterior end, of the ejaculatory duct, there is a papilla (*pa.*, Fig. 38) traversed by a funnel-like canal for the passage of spermatozoa from the vas deferens. In *fimbriata* the papilla is surrounded by a large bulbus propulsorius (*bu.pr.*, Fig. 38) about twice the diameter of the rest of the duct. In fifteen specimens the bulb measured 288 (165–415)  $\mu$  long by 249 (165–310)  $\mu$  in diameter. In *G. urna* the posterior end of the ejaculatory duct is not differentiated into a muscular bulb.

In *G. fimbriata* the vas deferens is of greater diameter, longer, more convoluted and much more capacious than in *urna*. In *G. urna* the anterior end of the vas deferens expands into a large ampulla (*a.v.d.*, Fig. 39) which may be mistaken for a bulbus propulsorius if its true position and relations with reference to the thick-walled ejaculatory duct are not noted. The epithelium lining the ampulla appears to be ciliated; possibly it should be considered to be the dilated posterior end of the valve-like papilla.

10. *The vagina and the vaginal pore.*—In *G. fimbriata* the thickened anterior part of the vagina is 1.5 (1.0–3.1) mm; in *urna* 1.9 (1.0–3.0) mm in length (average of 20 specimens of each species). This cuticle-lined, muscular section of the vagina

is usually more funnel-like in *fimbriata*; i.e., wider at its distal and more tapering toward the posterior end than in *urna* in which it is more slender and of more uniform diameter.

In *G. fimbriata* the vaginal pore (*p.vag.*, Fig. 2) is 10.7 (7.5–15) per cent; in *G. urna* 9.4 (7.1–14.1) per cent of the total length of the worm from the anterior end. As with the male pore, it is relatively closer to the anterior end in large than in small specimens. The vaginal pore is relatively closer to the lateral border in *fimbriata* than in *urna*.

11. *Vitelline glands*.—The vitelline follicles are absent, or very sparse, in the median part of the body overlying the central axis of reproductive organs. Anteriorly they are found on each side of the acetabulum, where they occur a little farther forward than the testes. From here they extend posteriorly, lateral to the axis of sex organs, both in the thicker median and the thinner lateral portions of the body, to the region between the level of the ovary and dorsal pore where they encircle the body more or less completely, although sometimes sparse medially on both dorsal and ventral sides. Throughout they are more numerous and crowded in the lateral margins, and become more widely spaced medially. The following specific difference is easily noted: In *G. urna* the vitelline follicles terminate posteriorly between the ovary and the dorsal pore of the funnel (*vit.*, Fig. 2). In *G. fimbriata* they also stop anterior to the dorsal pore medially, but extend laterally as far as the lateral ruffles; i.e., in mature specimens 1 or 2 mm posterior to the dorsal pore, on each side of the funnel (*vit.*, Fig. 1). No exceptions to this specific difference have been noted.

12. *Eggs*.—The eggs (Figs. 30–31) are plumply ellipsoid with bluntly rounded ends. Often the operculate end is slightly blunter than the other. In freshly deposited eggs the operculum cannot be seen, although the suture can be revealed by the application of acetocarmine, and it often can be detected in eggs which have stood in a salt solution for several days. The thin amber-colored shell is surrounded by a layer of jelly which remains invisible unless India ink is mixed with the medium in which the eggs are being examined. About 10 per cent of the eggs have a minute boss at the end opposite the operculum. As compared with those of *G. fimbriata*, the eggs of *G. urna* are slightly more pointed at the ends, i.e., shaped more like a Rugby football; have slightly thinner shells, and a slightly thicker layer of jelly. However, the differences between the eggs of the two species are so trivial that they cannot be readily used for specific determination.

Of the specific differences listed above, the relative lengths of the testicular fields, the size and complexity of the rosette, the distribution and appearance of the body spines, the size and grouping of acetabular spines (at least in *G. urna magnispinosa*), the size and appearance of vas deferens and ejaculatory duct, and the posterior limit of the vitelline glands are without interspecific gradations. Although their ranges overlap, the remaining characters are useful in the identification of specimens. In the opinion of the writer, it is not possible to confuse the species of *well-extended* preserved specimens, although the extreme mobility and contractility of the living worms result in occasional perplexity on the part of the observer.

#### VI. DISPUTED POINTS IN THE MORPHOLOGY OF *Gyrocotyle*

1. *Excretory pores*.—The excretory pores are visible in 86 per cent of my whole mounts of *G. urna* and in 78 per cent of those of *G. fimbriata*. Not only the pores,

but the large excretory vessels leading to them are often visible. The pores are easily found in serial cross-sections of either species. Spencer (1889) was the first to observe the true excretory pores. His illustration correctly depicts their location. Linton (1924) found the excretory pores and localized them correctly. Fuhrmann (1930-31, p. 161 and 171) states that the excretory pores are *ventral*, but otherwise localizes them correctly. Since Spencer's original discovery Lönnberg (1891), Hungerbühler (1910) and Watson (1911) were unable to find the pores. Dollfus (1923, p. 205, footnote 3 and p. 210, footnote 1) even doubts Spencer's observation and tries to homologize the acetabulum with the posterior excretory pore of the Digenea and certain Cestodes.

2. *Origin of the funnel by closure of the borders of a groove.*—Lönnberg (1891, p. 29) advanced the theory that the funnel, with its distal and proximal openings, was formed phylogenetically by the dorsal approximation and partial fusion of the lips of an elongated groove such as the bothrium of a Pseudophyllidean Cestode. Watson (1911, p. 428) seems to accept this hypothesis as a demonstrated fact. Ward (1912, p. 731) likewise appears to accept the theory as a fact and even describes the rosette as having a bilateral morphology, and states that the distal plicated border is horseshoe-shaped, with a ventral notch and furrow dividing it into a right and left series of ruffles continuous dorsally (Ward, 1912, p. 729 and p. 731).

My material of *Gyrocotyle fimbriata* includes numerous minute postlarvae in which the rosette is just beginning to appear, hundreds of juveniles with the rosette in all stages of development and adults of all sizes and degrees of maturity. There is no indication at any stage of a groove, the edges of which fuse to form a funnel. There is no sign, at any stage of development, of a bilateral arrangement of the plications of the rosette. In early stages of development the rosette has the form of a circular flange growing out from around the body just anterior to the hook-bearing process of the posterior end (Figs. 6-8). The border of the rosette remains circular until the development of plications begins to distort its symmetry, but at no time do the plications arrange themselves in any uniform or bilateral pattern, and at no time is the wall of the funnel divided longitudinally by any kind of notch or furrow. The dorsal pore appears as such, just anterior to the base of the papilla bearing the larval hooks, and has been discerned in a few specimens as small as 1.8-2.6 mm in length. Whatever its phylogenetic origin, there is nothing in the morphology or ontogeny of the funnel to support Lönnberg's theory.

3. *The genital notch (ins.gen., Figs. 1-2).*—The term "genital notch" has been applied to the indentation of the right margin which appears to be the termination of a groove proceeding from the vaginal pore. The notch is absent from 8 per cent of the preserved specimens of *G. fimbriata* and from 28 per cent of those of *G. urna*. It is usually deeper and more conspicuous in the former species. Ward (1912, p. 725) states that the genital notch occurs on both the right and left margins of the body. As a matter of fact, a scallop or indentation on the left side opposite the genital notch, appears to be merely a portion of the undulant margin, and not a counterpart of the genital notch, and such is entirely lacking from 66 per cent of the *G. fimbriata* and 44 per cent of the *G. urna* in the collection of the writer. In *urna* a distinct groove from the vaginal pore to the notch (or margin) is absent from 61 per cent, in *fimbriata* from 35 per cent, of the specimens.

4. *Suppression of lateral ruffles and rosette by muscular action.*—Lönnberg



(1891) describes *G. urna* as elongating to the extent that the lateral undulations completely disappear, and even the plications of the rosette in extremely elongated specimens. Olsson (1896) depicts specimens in the same extended state without obvious lateral undulations or rosette. Fuhrmann (1930-31, p. 170) states that the lateral ruffles are formed mechanically by muscular contraction. Dollfus (1923, p. 232, footnote 2) affirms that the plications of rosette and body margins may disappear completely in movements of elongation. Ward (1912, p. 727-728) states that the extent and complexity of the lateral ruffles depend on the state of contraction, and implies that they may disappear completely upon elongation of the worm.

The writer has observed small, scarcely mature specimens of *G. urna* elongate themselves to such an extent that the lateral undulations completely disappear, but without complete disappearance of the folds of the rosette. The observations of Lönnberg and Olsson are probably correct, but it is likely that they apply only to small specimens. The writer doubts that the lateral undulations of a mature, fully grown *G. urna* can be suppressed by elongation of the body, and feels certain that full-grown *G. fimbriata* is incapable of eliminating the folds of the lateral margins, or of the rosette, by any degree of extension.

5. *The male reproductive organs* (Figs. 38-39).—Lönnberg (1891) states that the ejaculatory duct has a ciliated lining except in the terminal part. Watson (1911, p. 379) states that the ejaculatory duct is lined with spinules. Haswell (1902) states that the ejaculatory duct is lined with chitinous denticles in *G. urna* and *G. nigrosetosa*.

The nature of the lining of the ejaculatory duct both in *G. fimbriata* and in *G. urna* is as follows: Beginning at the posterior end, the flattened epithelium covering the papilla and lining the muscular bulb, or fundus of the duct, is ciliated. The ciliated epithelium extends through the canal of the papilla in both species and into the terminal ampulla of the vas deferens in *urna*. The middle portion of the duct, which receives the secretion of the innumerable prostate gland cells, is lined by a low columnar epithelium which is not ciliated although the cells are vacuolated and the borders often frayed or seemingly macerated (coagulated secretion of the prostate gland?). Approximately the distal fifth of the ejaculatory duct is lined by cuticle continuous with that of the surface of the body. No spinules are present.

Spencer (1889) described the male pore as opening close to the margin on a conical papilla. He thought the ejaculatory duct is eversible. Although he called his specimens *G. urna*, his description was probably based upon *G. rugosa*.

Haswell (1902) stated that the male pore of his specimens of *G. rugosa* was situated on the apex of a papilla large enough to be curved around the margin of the body to the dorsal side, but that a similar papilla was not obvious in *G. urna*.

Lönnberg (1891) looked upon the proximal papilla ("Zapfen") as a valve to prevent the sperm discharged into the ejaculatory duct from returning to the vas deferens. He termed the entire ejaculatory duct the "penis apparatus" and stated that the external aperture lies on a papilla in a circular depression of the ventral surface. He thought that the terminal papilla functions as a penis, and that the terminal part of the ejaculatory duct is evaginable.

Watson (1911) designated the papilla at the posterior end of the ejaculatory duct the "penis papilla," and the ejaculatory duct the "cirrus pouch." She found the male pore to be located on a rounded papilla. She made no surmises regarding the functions of either papilla.



Fuhrmann (1930-31) looked upon the posterior papilla as a penis and appeared to think that the entire ductus ejaculatorius can be everted with the proximal papilla at its apex. He compares it with the protrusible penis of the TURBELLARIA and the MONOGENEA.

Here are two contrary opinions: one that the papilla at the proximal end of the ejaculatory duct is a protrusible intromittent organ, the other that the distal or terminal papilla bearing the genital pore is an intromittent organ. In the hundreds of specimens examined by the writer there is no evidence that the valvelike papilla in the fundus of the ejaculatory duct is ever protruded, or that it can even approximate the external opening, and no specimen was seen with the slightest eversion of the ejaculatory duct. The proximal part of the cuticle-lined terminal portion is sometimes prolapsed into the wider opening at the surface, thus forming a papilla (Fig. 39). There appears to be no permanently differentiated penis, although it is likely that the end of the ejaculatory duct and the surrounding surface may be protruded to form a temporary intromittent organ.

6. *The female genital complex.*—Apparently only Spencer (1889), Hungerbühler (1910) and Watson (1911) have attempted a complete analysis of the female reproductive system. Spencer's diagram omits the shell gland, yolk reservoir and ovicapt, and is excessively schematized. Hungerbühler presents a diagram which is essentially correct, but so schematized that it fails to show the true shape and proportions of the organs involved, and from which the ovicapt and shell gland are omitted. He erroneously states that the ductus seminalis is connected with the egg receptacle. Watson describes the female organs at length, but fails to describe the ovicapt, depicts the yolk reservoir with entirely erroneous shape and relations, and neglects to state where the ducts of the shell gland terminate.

*Description of the Female Genital System in*

*G. urna and G. fimbriata*

(Figs. 36-37)

The vagina extends from the vaginal pore, located anteriorly and dorsally, near the right border, down the right side of the uterus to open ventrally on the right anterior, or rarely the left anterior, side of the receptaculum seminis. In large specimens with highly developed uterine coils the vagina may be threaded between the loops of the uterus and be difficult to trace, even in serial sections. The anterior 5 to 13 per cent of the vagina in *fimbriata*, or 5 to 18 per cent in *urna*, depending on the degree of contraction or extension, is thick-walled and lined throughout by cuticle. A very narrow canal connects its lumen with the remaining thin-walled part of the vagina which is lined by a low, nearly cuboidal epithelium, which is ciliated in its posterior fourth.

The receptaculum seminis is nearly spherical, although usually longer than broad, and slightly flattened dorsoventrally. In mature worms its longitudinal and transverse diameters range from  $0.45 \times 0.37$  mm to  $2.2 \times 2.0$  mm. It is crowded with spermatozoa, and often contains scattering ova and vitelline cells. In immature specimens it is smaller, and often appears like a pyriform enlargement of the posterior end of the vagina.

The afferent oviducts, coming from the horseshoe-shaped aggregation of ovarian follicles, converge to a receptaculum ovorum on the ventral side of the seminal

receptacle. The number and arrangement of oviducts is inconstant. The egg receptacle is dorso-ventrally flattened, transversely elongated, and is of various sizes and shapes in different specimens. In large worms of either species it may be from 300 to 700  $\mu$  in transverse diameter and from 100 to 200  $\mu$  in antero-posterior diameter. Its posteromedian border opens into a conspicuous muscular ovicapt which continues posteriorly as the ciliated efferent oviduct, or fertilization canal. The short ductus seminalis, coming from the seminal receptacle, unites with the efferent oviduct just distally to the ovicapt. Typically, the ductus seminalis lies dorsally to the ovicapt; their superimposed position often results in the two ducts appearing as one in whole mounts or frontal sections.

The yolk reservoir empties into the oviduct just distal to the junction of the seminal duct therewith. The yolk reservoir is extremely conspicuous; indeed, it was correctly described and illustrated by Wagener (1852). Its shape is that of a spindle bent into a crescent, or a short helicoid curve, with a variable number of yolk ducts emptying into its more anterior and ventral end. It is invariably crowded with opaque yolk cells. It varies from 200 to 700  $\mu$  in length by 70 to 100  $\mu$  in greatest diameter. After its junction with the yolk reservoir, the oviduct begins to curve, usually to the left, occasionally to the right (where the vagina enters the seminal receptacle on the left side), and enlarges somewhat both in the diameter of the lumen and the thickness of the walls. At this point, near the posterior boundary of the seminal receptacle, the ducts of the shell gland penetrate the walls of the oviduct. This portion of the oviduct has all the characteristics of an oötype, and is here considered to be such, although it is invariably empty in preserved specimens.

Distal to the oötype the oviduct, now better termed the uterine duct, contracts a little in diameter and continues anteriorly in a sinuous course to the anterior boundary of the seminal receptacle, where it becomes so excessively coiled and intertwined that its true length is hard to judge. The proximal part contains scattering ova, yolk cells and secretory globules; the more convoluted distal portions fully formed eggs. As the eggs accumulate, the uterine duct rather quickly enlarges, loses its cilia, and passes over into the uterus.

The fact that in my preparations the oötype is invariably empty, and the proximal parts of the uterine duct contain only the constituent elements which go to form the compound egg, at first led to the supposition that the egg is actually formed in the more distal parts of the uterine duct. Watson (1911, p. 397) says, "The first two or three coils of the uterus . . . may be regarded as an oötype . . .," meaning, apparently, that the entire proximal portion of the uterine duct functions as an oötype. She also observed fully formed eggs only in the convolutions of the uterus anterior to the receptaculum seminis. The probable explanation of the emptiness of the oötype and proximal part of the uterine duct is that the worms are usually fixed hours, or days, after removal from the host and that normal egg production had long since ceased.

In both species the uterus consists of two parts, a proximal spirally coiled portion and a terminal saccate portion, for convenience designated the *uterus* and *uterine sac*. The uterine sac comprises 35 (24-54) per cent of the total length of the uterus. The uterine pore opens in the mid-ventral line a short distance back of the anterior end of the saccate uterus. It is often invisible in small mature worms, and probably becomes patent only after a large quantity of eggs has accumulated. The uterus

and uterine sac are relatively bulkier, with a greater volume of eggs, in *G. fimbriata*, but otherwise there is no distinct difference in the morphology or proportions in the two species. Among previous students of the genus, only Watson seems to have distinguished clearly the uterine sac as distinct from the rest of the uterus.

7. *The nervous system.*—Only Watson (1911) has attempted a detailed analysis of the nervous system of *Gyrocotyle*. The writer will only present a few observations which differ from the interpretations of Watson.

Miss Watson describes the two large posterior ganglia ("ganglionic knots") as connected by a "posterior bridge commissure." Each ganglion is said to divide at its posterior end into dorsal and ventral branches which pass medially to join similar branches from the opposite side to form a "proximal ring commissure." Further posteriorly, near the base of the rosette she found a "distal ring commissure" connected with the "proximal ring commissure" by four connectives, two from the dorsal and two from the ventral portions thereof, and with the ganglia by a single nerve on each side which bifurcates before uniting with the "distal ring commissure."

Fig. 35 is a drawing of the posterior ganglia and commissures from a whole mount of *G. urna*. The same pattern is found in *G. fimbriata*. It will be noticed that the posterior transverse commissure (Fig. 35, *comm.p.*), between the anterior ends of the two large posterior ganglia, bears a pair of distinct commissural ganglia not mentioned by Watson. At their posterior ends, the two main ganglia divide each into three branches (Fig. 35, *ra.a.*, *ra.m.*, *ra.v.*) of which the dorsal and ventral ones proceed medially and anastomose with the branch from the opposite side, while giving off numerous anastomosing twigs towards the posterior end. The posterolateral ramus proceeds much further posteriorly before dividing into ramifying twigs.

Watson's description of the posterior ganglia and commissures is correct in the main, but deficient or obscure in the following details: 1. She failed to observe the two ganglia of the posterior commissure. 2. She implies that there is a continuous ring commissure near the ganglia, although in reality she describes dorsal and ventral semicircular commissures. Actually, there is no continuous "proximal ring commissure." 3. Her drawing (Watson, 1911, pl. 35, fig. 17) is obscure and does not show the posterolateral rami as distinct from the dorsal and ventral posterior rami. 4. The distal ring commissure described by Watson cannot be seen in whole mounts and I have been unable to trace it in serial sections. The numerous subdivisions of the three posterior rami proceeding from each ganglion anastomose to form an elaborate network, which encircles the posterior part of the funnel region, and extends into the plications where its ultimate ramifications cannot be traced. A well-defined commissure, sharply delimited from the network, and forming a continuous ring proximal to the folds of the rosette, obviously does not exist.

The writer has been unable to discern the sensory ridges and sensory pits of the acetabulum, described by Watson, in living worms, whole mounts or in serial sections.

#### VII. LIFE HISTORY

Since *Gyrocotyle* is a parasitic Platyhelminth and has been regarded both as a Trematode and a Cestode, it has generally been assumed that it has one or more intermediate hosts. The manner in which the Chimaerid fishes receive their infestations is still unknown. Except for *G. rugosa*, the eggs of which contain a fully



developed larva at the time of deposition, the eggs are discharged in early embryonic stages and must undergo several weeks of development before hatching.

Ruszkowski (1932) and the writer have both kept eggs in dishes of sea water until the free-swimming larvae hatch, so it is logical to believe that in nature the larvae develop in eggs which have passed out of the intestine of the host and that they penetrate some intermediate host on the ocean floor, although this belief is without supporting evidence.

Fig. 5 is a free-hand drawing of an actively swimming decacanth larva of *G. fimbriata*. Most swimming specimens are slightly curved toward one (the ventral?) side. As Ruszkowski pointed out, the larvae swim with the openings of the glandular ducts at the anterior end and with the cluster of larval hooks at the posterior end. Since the rosette and funnel develop at the end with the larval hooks, he was able definitively to settle the oft-debated question as to which end of the adult actually is anterior.

It is extremely difficult to get exact measurements of the living larvae of *Gyrocotyle* and to analyze the internal structures. When in good condition they are in constant, rapid motion. If restrained in a hanging drop or under a cover glass they quickly contract into various spheroid, ovoid, or irregular shapes and soon the entire anterior half disintegrates into a mass of globules and protoplasmic debris. In most fixing fluids they contract to an ovoid shape. Specimens discharged from a pipette into a mixture of five parts of 6 per cent formalin and one part of 2 per cent osmic acid contracted only slightly, and died at an average length of  $140\ \mu$  by  $40\ \mu$  in maximum diameter. Normal living specimens have about the same length, but seem more slender. The larvae are extremely contractile and extensible, and normally active ones may elongate to about  $170\ \mu$  with a diameter of about  $20\ \mu$ , or may contract to dimensions of about  $100\ \mu$  by  $30\text{--}40\ \mu$ .

Typically, they have a short fusiform shape, with the greatest width from 20 to 50 per cent of the total length from the anterior end. The entire larva, except for the extreme anterior end, is covered with a dense layer of cilia which, when actively beating, form a layer about  $5\ \mu$  thick. In dead or moribund specimens the cilia stand out at right angles to the surface and appear to be about  $11\ \mu$  long, except near the anterior end where they become progressively shorter and disappear. The anterior end, like the corresponding area of a miracidium, is devoid of cilia and is protrusible and retractile. The cilia arise from a thin membranous epithelium about one micron thick, in which scattered nuclei are present, although cell boundaries could not be distinguished. Immediately beneath the epithelium are circular myonemes, more numerous anteriorly than posteriorly.

Terminating at the anterior end are two long ducts which stain intensely with neutral red or Nile blue A and which appear to be filled with a nearly solid secretion. They can be traced posteriorly about two-thirds the length of the larva, but become indistinct and fade from view just anterior to a mass of vaguely outlined cells located in front of the larval hooks. The two ducts are closer to one surface (arbitrarily considered dorsal) than to the other. At the anterior end they seem to terminate separately in some, and to unite in other, larvae. A short distance back from the anterior end is a large bilobed mass of cells which may possibly be the "brain" or Anlage of the nervous system. The parenchyma of the entire larva contains numerous refractive globules, mostly from 2 to  $5\ \mu$ , but ranging from less than



one to  $10\ \mu$  in diameter. These globules are clustered in large numbers ventral to the "brain" and near the posterior end of the secretory ducts. Occasionally, two small transient clear vesicles, right and left, could be seen anterior and lateral to the "brain"; possibly contractile excretory vesicles.

The ten larval hooks are disposed in a circle at the posterior end, completely retracted within the body, with the tips turned inward and the manubria diverging. The hooks are protruded so infrequently that an exact analysis of the process was impossible. Apparently, the ciliated integument is retracted at the same time that a pluglike papilla bearing the hooks is protruded. The curved tips of the hooks thus appear spread around the periphery of the papilla, with the manubria converging toward the longitudinal axis of the larva. Upon withdrawal of the papilla, the tips close in toward the center.

The larval hooks (Figs. 32–34) are  $21\ (19\text{--}22)\ \mu$  in length. They persist in postlarval stages at the free end of a pluglike papilla and apparently are functional in the smaller juvenile specimens. After the formation of the dorsal pore and funnel, the hooks become incorporated in the dorsal wall of the funnel where they occasionally can be seen in fully mature specimens. In fact, they can clearly be seen in one of my whole mounts of *G. urna* 32 mm in length.

The following zoologists have reported finding postlarvae in various stages of development in the parenchyma of larger specimens of *Gyrocotyle*: Hungerbühler (1910) in *G. rugosa* and *G. urna*; Linton (1924) in *G. plana* (= *G. rugosa*), and Fuhrmann (1930–31) in *G. urna* and *Gyrocotylodes nybelini*.

In the course of this study such embedded larvae have been found in the parenchyma of 15 adult specimens of *G. urna*, in 23 adults of *G. fimbriata* and in 6 small juvenile *fimbriata* ranging from 0.9 to 7.0 mm in length. The embedded postlarvae measure from  $110 \times 50\ \mu$  (scarcely bulkier than a free-swimming larva) to  $1800 \times 380\ \mu$ . They lie at various depths beneath the surface, in any part of the host, but are commonest just anterior to the uterine sac and in the vicinity of the male genital pore.

In two specimens of immature *G. urna*, the uterine sac (as yet devoid of eggs) was occupied by a juvenile *Gyrocotyle*, one 2.1 mm long, the other 6.9 mm long—longer than the uterine sac and consequently folded back on itself at either end. Since an open uterine pore could not be discovered in either case, it is likely that the ensconced juveniles had developed to their relatively large size in their actual location.

Immediately in front of the uterine sac, on the ventral surface of the worm, is a subcircular area of closely crowded nuclei, 250 to  $500\ \mu$  in diameter, with thickened cuticle, which usually has a depression in its center. It is more conspicuous and well defined in *G. urna* than in *G. fimbriata*. Of the 40 mature specimens of both species with embedded postlarvae, 23 had all, or part, of the postlarvae lodged beneath, or adjacent to, this spot. Only Wagener (1852, p. 552) seems to have noted the spot. Since its exact function is unknown, it is given the non-committal name of *fovea Wageneri* in this paper (*fov.wag.*, Figs. 1 and 2).

The maximum number of postlarvae found in any one host was 101, in a specimen of *G. fimbriata* 26 mm long. Usually, only from 1 to 8 are present; in fact, 76 per cent of the infected host animals harbor only one parasitic postlarva.

The considerable frequency with which normally developing postlarvae are

encountered in the tissues of larger *Gyrocotyle*, which thus function as nurse animals, suggests that this is not an abnormal or chance occurrence, but a normal phase in the life history of the genus. On the other hand, as Fuhrmann suggested, they may normally pass through early postlarval stages in the intestinal mucosa of the ratfish and only enter the larger *Gyrocotyle* incidentally, or under abnormal conditions.

#### VIII. PERCENTAGE OF INFESTATION AND THE NUMBER OF *Gyrocotyle* IN A SINGLE HOST

Most writers on *Gyrocotyle* have reported that there normally are only one or two worms in a single host, although rarely several may be found. My records do not indicate precisely the percentage of infestation nor the correct number of worms in the host in every case, since often worms escape from the host before it can be examined for parasites. In the course of this study, 20 specimens of *Gyrocotyle* have been collected from the bottom of containers in which ratfishes were transported. The following figures are of some significance, however, in view of the large number of ratfishes examined.

Data are available for 167 ratfishes. Of these, 24 were negative for *Gyrocotyle*, although possibly infested when first captured. Of the 143 infested ratfishes, 3 harbored *G. fimbriata* and *G. urna* simultaneously; 104 had *G. fimbriata* only and 36 had *G. urna* only.

Of the 104 ratfishes with *G. fimbriata*, 16 had one worm; 70 had two; 6 had three; 1 had five; 4 had six; and 7 had mass infestations of large numbers of small juvenile worms, ranging from 7 to 203 in a host.

Of the 36 ratfishes with *G. urna*, 8 had one worm; 26 had two, and 2 had three.

These records agree with the experiences of others, and show that the ratfish usually has two mature *Gyrocotyle*, of one species, in the spiral valve. The manner in which the large number of juvenile worms sometimes present is reduced to several adults, or how the presence of one species excludes the other, remains to be elucidated.

#### IX. THE TAXONOMIC POSITION OF THE GENUS *Gyrocotyle*

*Gyrocotyle* is classified as a Cestode primarily because of the lack of a digestive tract, although the pattern of the reproductive system, with its large ovicapt and long uterine duct, bears some resemblance to that of the TETRAHYNCHIDEA. On the other hand, the anterior sucker and posterior haptor, the position of the excretory pores, the anteroventral uterine and male genital pores, and the dorsal vaginal pore (cf. *Microcotyle*, *Hexacotyle*, etc.) lend it the superficial appearance of a Monogenetic Trematode. There obviously is no series of morphologically different larval stages strictly comparable to the procercooids, plerocercoids, etc., of Cestodes, and as yet no positive evidence that intermediate hosts are involved in the life cycle. The free-swimming larva resembles no known cestode larva with the possible exception of the poorly described larva of the Amphilinidae. To judge from the postlarval stages found in the intestine of the ratfish, or embedded in the tissues of adult *Gyrocotyle*, the metamorphosis from the larval to the adult stage is extremely simple and direct, and much more comparable to that of the Monogenea than to that of the Cestoda. Until the life history of the genus has been completely elucidated its taxo-

onomic position will remain obscure. It can be considered a Cestode only provisionally.

#### X. THE VALID SPECIES OF *Gyrocotyle*

The reader is referred to the works of Watson (1911) and Dollfus (1923) for a review of the complicated bibliography of *Gyrocotyle* and summaries of the contents of early publications.

*Gyrocotyle rugosa* Diesing, 1850; the type species.—The first species named was *G. rugosa*, described by Diesing in 1850, and redescribed with illustrations in 1855. His description, confined to the external morphology, indicates a worm without lateral undulations, but with a fairly complicated rosette. There is no mention of spines. Diesing was in error as regards the host which was discovered by Monticelli (1889) to be *Callorhynchus antarcticus*. Descriptions of *G. rugosa* remain incomplete and unsatisfactory to the present day.

Spencer (1889) described what he believed to be *G. urna* on the basis of four specimens from *C. antarcticus*. A study of his description shows that he published a composite description of one specimen of *G. rugosa* and of three others which possibly were *G. urna*. He gives no measurements and his more important drawings apparently are of compressed and distorted specimens. He depicts the male genital pore as marginal and the uterine pore to the right of the median line.

O. v. Linstow (1901) published a brief description of a single specimen of *G. rugosa*, but was in error as regards the host. His specimen had no lateral undulations, and he saw no spines. The eggs contained a larva with 10 hooks.

Haswell (1902) added some data on *G. rugosa*. He stated that the male pore is marginal or submarginal, with a protrusible penis; that the vaginal aperture is dorsal, to the right of the median line, but posterior to the male opening. He mentioned the "embryo" with large hooks inside the egg. He did not state the host of his specimen.

Hungerbühler (1910) had seven specimens from *C. antarcticus*. He mentions lengths of 42 to 96 mm, gives measurements of breadth, thickness and width of rosette, states that the lateral margins are smooth, that transverse rugae encircle the body and that the uterus opening is median. He does not localize the male and vaginal pores precisely, but the male pore is apparently closer to the margin than in other species, at the apex of a well-developed papilla. The acetabular spines of his specimens are 200 to 300  $\mu$  long by 20  $\mu$  wide. Body spines were not observed. The egg contains a 10-hooked larva.

Linton (1924) described two specimens from *C. antarcticus* which he designated *G. plana* n. sp., but which appear to have been *G. rugosa* (this opinion was first expressed by Johnston, 1934). He described the margins as finely crenulated, but not ruffled. He observed only acetabular spines, 400  $\mu$  long by 48  $\mu$  in diameter. The male genital pore was a little to the right of the median line on a mound-like elevation, the genital and uterine pores being arranged as in *G. urna*. Testes extended 23 per cent of the total length of the worm in one specimen. Eggs in the terminal part of the uterus contained 10-hooked larvae. Only the uterine structure (a central axis with lateral diverticula) seems to distinguish this species from *G. rugosa*, and this feature probably was a misinterpretation of the uterus in strongly compressed and poorly preserved specimens.



MacDonagh (1927) briefly described two specimens of *G. rugosa* from *C. antarcticus*, fixed under pressure. Aside from measurements, his descriptions state little beyond the fact that the lateral undulations are absent, the folds of the rosette relatively simple, and spines apparently lacking.

*G. rugosa* undoubtedly is a distinct species. All the existing descriptions are inadequate or based on distorted specimens, and the only characters which seem to set it off clearly from other known species are the thin-shelled eggs containing a fully developed decacanth larva prior to deposition, the lack of definite ruffles on the lateral margins, the relatively smaller rosette, and the lack of body spines. The existent data on such characters as the relative position of the genital pores and the supposedly large male sex papilla are confusing and contradictory.

By combining the consistent features of the several descriptions, and omitting contradictory or dubious data, a provisional, although incomplete, diagnosis of the species can be assembled:

*Gyrocotyle rugosa* Diesing, 1850

*Synonymy*: *Amphityches rugosa* Spencer, 1889; *Gyrocotyle plana* Linton, 1924.

*Diagnosis*: With the characters of the genus. Mature specimens 42 to 96 mm long. Rosette relatively small and simple, about one-third (21–38 per cent) of greatest diameter of the body. Margins of the body without undulations, but transverse rugae encircle the body producing marginal crenulations. Acetabular spines 200–400  $\mu$  long by 20–48  $\mu$  in diameter; body spines probably absent. Testicular areas about 23 per cent of total length of worm. Male genital pore ventral, distinctly to right of median line, usually on a distinct papilla. The genital pores and uterine pore apparently closer to the anterior end than in other species. Eggs very thin-shelled, 96–130  $\mu \times$  52–65  $\mu$ , containing a fully developed decacanth larva at time of deposition.

*Host*: *Callorhynchus antarcticus* Lacépède.

*The species of Gyrocotyle from Chimaera monstrosa*.—An examination of the publications of European writers who have described *G. urna* from *Chimaera monstrosa* is enough to convince one that they were dealing with two distinct species. In fact, Prof. Collet of Christiania (*in* Lönnberg, 1891, p. 17) states that he believes there are two species in *Ch. monstrosa*. Lönnberg (1891) mentions a flat *Ligula*-like form and a ruffled form, but regards them as different stages in the contraction of the same species.

In spite of the voluminous European literature on *G. urna*, none of the authors has presented really good drawings of the worm, none of the descriptions contains adequate measurements of the spines, if, indeed, any measurements are given, and the distribution of the spines is usually insufficiently analyzed. The extents of the areas of testicular and vitelline follicles are usually ignored or only vaguely described. Only Wagener (1852) mentions the length of the testicular areas relative to the total length of the worm, but with no indication of how many worms were so measured. Drawings of the rosette are often so poorly executed that it is impossible to determine whether it is of the *urna* or of the *fimbriata* type. No one gives the relative breadth of rosette to breadth of body, and no one presents data on the number of lateral ruffles and their amplitude. Only the following papers contain descriptions or illustrations of any value for judging the species of *Gyrocotyle* in *Ch. monstrosa*: (1) Wagener, 1852, pl. 14, fig. 1, *urna* type; pl. 14, fig. 3, *fimbriata* type; pl. 14, fig. 7, apparently a composite drawing, although possibly *urna*. (2) Krøyer, 1852–53, gives an inadequate description, applicable only to *G. fimbriata* as far as it goes. (3) Lönnberg, 1891, pl. 3, fig. 34, *urna* type; pl. 3, fig. 36, *urna* type;



pl. 3, fig. 37 has the proportions of a juvenile *G. fimbriata*. (4) Scott, 1911, published magnified photographs of one immature and three mature specimens, which all have the precise aspect and proportions of *G. fimbriata*. (5) Dollfus, 1923, published drawings (p. 238, fig. 7) labeled *G. urna* which actually are *G. fimbriata*. (6) Fuhrmann, 1930, published a drawing (p. 161, fig. 193, 1) of a contracted specimen designated *G. urna* but apparently *G. fimbriata*.

Since the original description of *G. urna* by Wagener is obviously a composite description of two species, the problem of deciding which should retain the original specific name arises. Watson (1911) in her description of *G. fimbriata* from *Hydrolagus collieri* points out the existence in the same host of another species which she considers to be *G. urna*. Since hers is the first description which differentiates between worms of the *fimbriata* and *urna* types, her allocation of these species should be allowed to stand, although the European descriptions and illustrations involve a worm of the *fimbriata* type probably more frequently than they do the species referred to *urna* by Watson.

*Gyrocotyle urna* from other hosts.—Haswell (1902) described *G. nigrosetosa* from *Chimaera ogilbyi* Waite. The color of the spines, as pointed out above, cannot be considered a specific character. The description is inadequate for specific recognition, but such as it is, it agrees fairly well with the morphology of *G. urna*. Yamaguti (1934) reports *G. urna* from the same host. His identification is probably correct, although the description is insufficient for positive recognition. It seems highly probable that *G. urna* occurs in *Chimaera ogilbyi*.

Spencer (1889) and Hungerbühler (1910) described specimens from *Calorhynchus antarcticus* which were designated *G. urna*. The descriptions are inadequate and the presence of *G. urna* in this host is in need of further investigation.

#### XI. SPECIES INQUIRENDAE

*Gyrocotyloides nybelini* Fuhrmann, 1930. At first impression this species appears to constitute a distinct genus. It differs from *Gyrocotyle*, according to Fuhrmann, in lacking spines and lateral undulations, in having the funnel region extremely elongated and without a plicated border, in having a mere pit in place of the muscular acetabulum, testicular fields which extend back to the ovary and paired excretory pores on the lateral margins. Fuhrmann considers the elongated forms of *G. urna* described by Lönnberg to belong to his new genus.

However, Fuhrmann presents no genuine diagnosis of the genus or species. He gives no measurements of any kind, no description or drawing of the egg, and no adequate statement of the proportions and location of many internal structures. The one detailed drawing (p. 164, fig. 196) is obviously an immature specimen with the uterus still straight and undifferentiated, with the ejaculatory duct and male pore not clearly depicted, and with excretory pores, vitelline glands and nerve ganglia omitted. The number of specimens at his disposal is not stated. Thus, the description is incomplete and unconvincing. It is difficult to eliminate the suspicion that Fuhrmann was dealing with small, barely mature specimens of one of the species of *Gyrocotyle* in an unusual state of elongation.

*Gyrocotyle maxima* MacDonagh, 1927. The inadequate description, based on a single specimen, includes only a few external measurements. Spines were not observed. Eggs are as in *G. rugosa*. The host (*Mustelus asterias*) is probably incidental, since sharks are known to prey upon Chimaerids.

## XII. SUMMARY AND CONCLUSIONS

1. It is pointed out that *Chimaera monstrosa* harbors at least two species of *Gyrocotyle*, one of which Watson considered to be the original *G. urna* and one similar to her *G. fimbriata*. Existing descriptions by European zoologists are mostly composite, as well as inadequate, and fail to distinguish between the two. It is probable that the two species from *Hydrolagus colliei* are identical with those from *Ch. monstrosa*.
2. *Gyrocotyle fimbriata* Watson from *Hydrolagus colliei* is redescribed.
3. *Gyrocotyle urna* from *Hydrolagus colliei* is described, and two forms, differing in the size of the acetabular spines, are distinguished.
4. Previous descriptions of the male and female reproductive organs of *Gyrocotyle*, and of the posterior ganglia and commissures, are supplemented with added details.
5. Various controversial points in the morphology of *Gyrocotyle* are discussed and in part clarified.
6. The decacanth larva and early postlarval stages are described and illustrated. The frequent occurrence of developing postlarvae in the tissues of older individuals of the same species is noted. The existing evidence seems opposed to the theory that intermediate hosts are involved in the life cycle, although the complete life cycle remains unresolved.
7. *Gyrocotyle rugosa* is discussed and a provisional specific diagnosis is given.
8. Although existing data are inadequate for a positive decision, it is very likely that *G. urna* occurs in *Chimaera ogilbyi*. The existence of *G. urna* in *Callorhynchus antarcticus* is probable, but the data are even more unsatisfactory.
9. *Gyrocotyle maxima* MacDonagh and *Gyrocotylodes nybelini* Fuhrmann are so imperfectly described that their systematic status remains in question.

## REFERENCES

- DIESING, CARL M. 1850. *Systema Helminthum*. v. 1. Pp. xiii + 680. Vindobonae, Wilhelm Braumüller.
- DIESING, KARL M. 1855. Sechzehn Gattungen von Binnenwürmern und ihre Arten. Denkschr. Akad. Wiss. Wien, Math.-naturw. Classe 9(1): 171-185, pls. 1-6.
- DOLLFUS, R. PH. 1923. L'orientation morphologique des *Gyrocotyle* et des Cestodes en général. Bull. Soc. Zool. France 48: 205-242, 7 textfigs.
- FUHRMANN, O. 1930-31. Cestoidea. In Kükenthal und Krumbach. *Handbuch der Zoologie*. 2(Pt. 1): 141-416, 260 textfigs.
- GALIGHER, A. E. 1934. *The Essentials of Practical Microtechnique in Animal Biology*. Pp. 288, 58 figs. Berkeley, Calif. Pub. by author.
- HASWELL, W. A. 1902. On a *Gyrocotyle* from *Chimaera ogilbyi* and on *Gyrocotyle* in general. Proc. Linn. Soc. N. S. Wales 27(1): 48-54, pl. 7.
- HUNGERBÜHLER, MAX. 1910. *Studien an Gyrocotyle und Cestoden*. Pp. 4 + 26, 2 pls. Inaug.-Diss. Univ. Basel.
- JOHNSTON, T. H. 1934. Remarks on some Australian Cestodaria. Proc. Linn. Soc. N. S. Wales 59(1-2): 66-70.
- KRØYER, HENDRICK. 1852-53. *Danmarks Fiske*. 3(Pt. 2): [2] + 705-1279 + [2]. Copenhagen, S. Triers.
- LINSTOW, O. v. 1901. Entozoa des zoologischen Museums der Kaiserlichen Akademie der Wissenschaften zu St. Petersburg. Bull. Acad. Imp. Sci. St.-Petersbourg, V<sup>e</sup> Sér. 15(3): 271-292, pls. 1-2.
- LINTON, EDWIN. 1924. *Gyrocotyle plana* sp. nov. with notes on South African Cestodes of fishes. Union of So. Africa. Fisheries and Marine Biological Survey. Report No. 3. Spec. Rept. VIII: 1-27, pls. 1-7.
- LÖNNBERG, E. 1891. Anatomische Studien über skandinavische Cestoden. Kongl. Svenska Vetenskaps-Akademiens Handlingar. N. F. 24(Pt. 1; No. 6): 1-108, pls. 1-3.

- MACDONAGH, E. J. 1927 Parásitos de peces comestibles. III. Dos cestodarios: *Gyrocotyle rugosa* del "Pez gallo" y *Gyrocotyle maxima* n. sp. del "Gatuso." La Semana Médica (Buenos Aires) 34: 1232-1235, 9 textfigs.
- MONTICELLI, F. S. 1889 Notes on some entozoa in the collection of the British Museum. Proc. Zool. Soc. London 1889: 321-325, pl. 33.
- OLSSON, P. J. 1896 Sur *Chimaera monstrosa* et ses parasites. Mém. Soc. Zool. France 9: 499-512, 9 textfigs.
- RUSZKOWSKI J. S. 1932 Études sur le cycle évolutif et sur la structure des Cestodes de mer. IIIème partie. Sur les larves de *Gyrocotyle urna* (Gr. et Wagener). Bull. Internat. Acad. Polonaise Sci. et Lett. Sér. B, (II) 1931(7-10): 629-641, 2 textfigs.
- SCOTT, THOMAS 1911 Notes on some trematode parasites of fishes. Twenty-eighth Annual Report of the Fishery Board for Scotland (for 1909) 28(3): 68-72, pls. 7-8.
- SPENCER, W. BALDWIN 1889 The anatomy of *Amphiptyches urna* (Grube and Wagener). Trans. Roy. Soc. Victoria 1(2): 138-151, pls. 11-13.
- WAGENER, R. G. 1852 Ueber einen neuen in der *Chimaera monstrosa* gefundenen Eingeweide-Wurm, *Amphiptyches urna* Grube und Wagener. (Müller's) Archiv f. Anat. Physiol. u. wiss. Med. Jahrg. 1852: 543-554, pls. 14-15.
- WARD, H. B. 1912 Some points on the general anatomy of *Gyrocotyle*. Zool. Jahrbücher. Suppl. 15 2: 717-738, pl. 32.
- WARDLE, R. A. 1932 The Cestoda of Canadian fishes. I. The Pacific Coast Region. Contrib. Canad. Biol. and Fisheries, N. S. 7(18): 221-243, 15 figs.
- WATSON, EDNA E. 1911 The genus *Gyrocotyle* and its significance for problems of Cestode structure and phylogeny. Univ. Calif. Publ. Zool. 6(15): 353-468, pls. 38-48.
- YAMAGUTI, S. 1934 Studies on the helminth fauna of Japan. Part 4. Cestodes of fishes. Jap. Jour. Zool. 6(1): 1-112, 187 textfigs.

## EXPLANATION OF PLATES

Figs. 1 and 2 are drawings of stained whole mounts, slightly schematized as follows: (1) The mass of opaque eggs has been omitted from the uterus and uterine sac. (2) The testicular follicles are more numerous and crowded in both species than here depicted, although the relative appearance of the testicular fields in the two species is shown with approximate correctness. (3) The vitelline follicles are represented as clear circles. Each follicle is composed of a group of strongly staining cells crowded with yellowish yolk granules. Consequently, in stained preparations, the yolk follicles are semiopaque. (4) The spines, in haematoxylin-stained preparations, may vary from colorless to purple. They are here depicted as black to avoid confusion with adjacent structures.

Figs. 6-35, inclusive, were drawn with the aid of a camera lucida

## ABBREVIATIONS

AC. ....	acetabulum	PA. ....	proximal papilla of ejaculatory duct
A.V.P. ....	ampulla of vas deferens in <i>G. urna</i>	P.EJ. ....	male genital pore (ventral)
BU.PR. ....	bulbus propulsorius of ejaculatory duct in <i>G. fimbriata</i>	P.VAG. ....	vaginal pore (dorsal)
COMM.P. ....	posterior commissure, bearing two commissural ganglia	RA.D. ....	posterodorsal ramus from the posterior ganglion
CU. ....	cuticle	RA.M. ....	posterolateral ramus from the posterior ganglion
D.P. ....	dorsal pore of funnel	RA.V. ....	posteroventral ramus from the posterior ganglion
DU.EJ. ....	ejaculatory duct	REC.OV. ....	receptaculum ovarum
DU.OV. ....	oviduct	REC.SEM. ....	receptaculum seminis
DU.SEM. ....	ductus seminalis	REC.VIT. ....	yolk reservoir
DU.UT. ....	uterine duct	ROS. ....	rosette
DU.VIT. ....	vitelline duct	SH.GL. ....	shell gland
EX.P. ....	excretory pore	SP.AC. ....	acetabular spines
FOV.WAG. ....	fovea Wageneri (ventral)	SP.AL. ....	anterolateral spines
GA.A. ....	anterior ganglion	TE. ....	testicular follicles
GA.P. ....	posterior ganglion	UT. ....	uterus
GEL. ....	gelatinous layer surrounding shell of egg	UT.P. ....	uterine pore (ventral)
GL.PR. ....	prostate gland	UT.S. ....	uterine sac
INS.GEN. ....	genital notch	VAG. ....	vagina (slender portion)
M.R. ....	radial muscles of ejaculatory duct	VAG.CR. ....	vagina (thickened terminal section)
NEL. ....	longitudinal nerve trunk	V.D. ....	vas deferens
OVC. ....	ovicapt	VIT. ....	posterior limit of vitelline follicles
OOT. ....	oötype		
OVAR. ....	ovary		



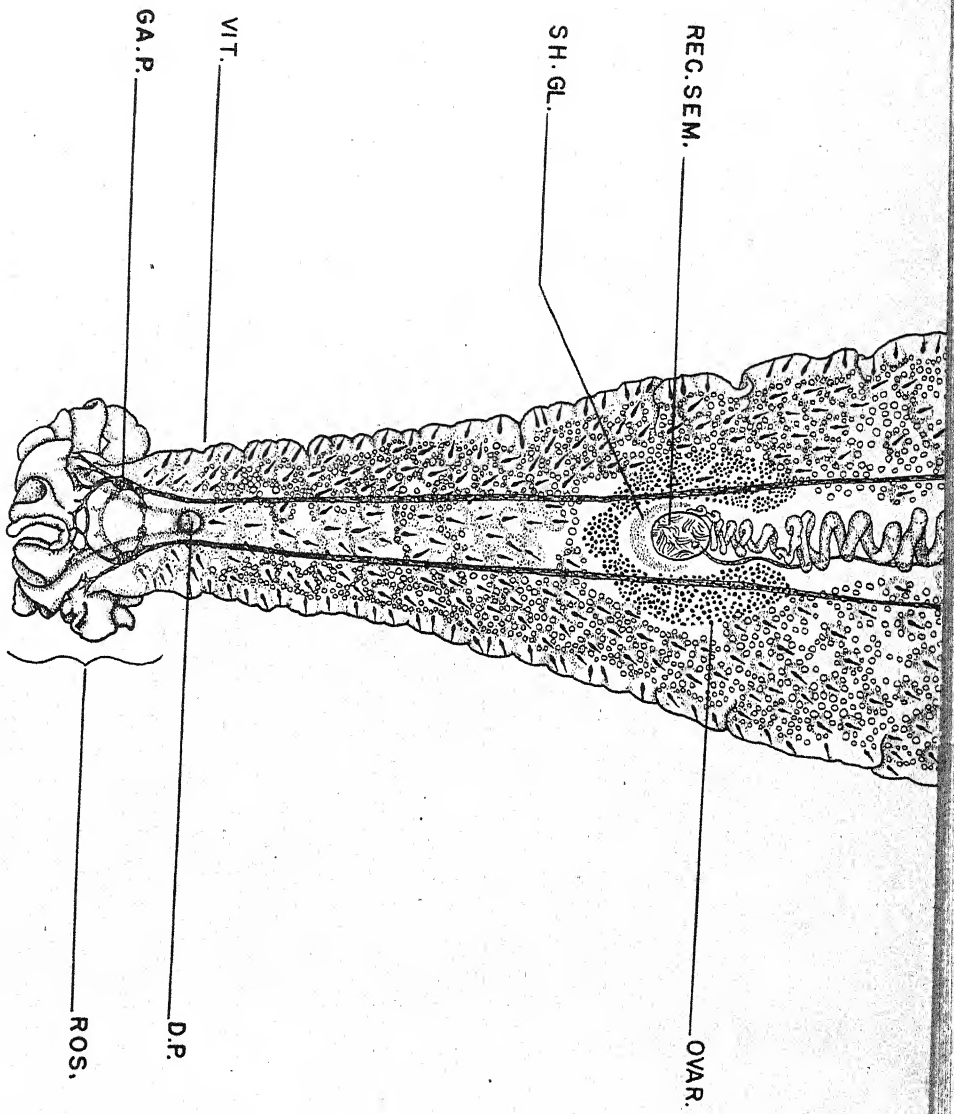
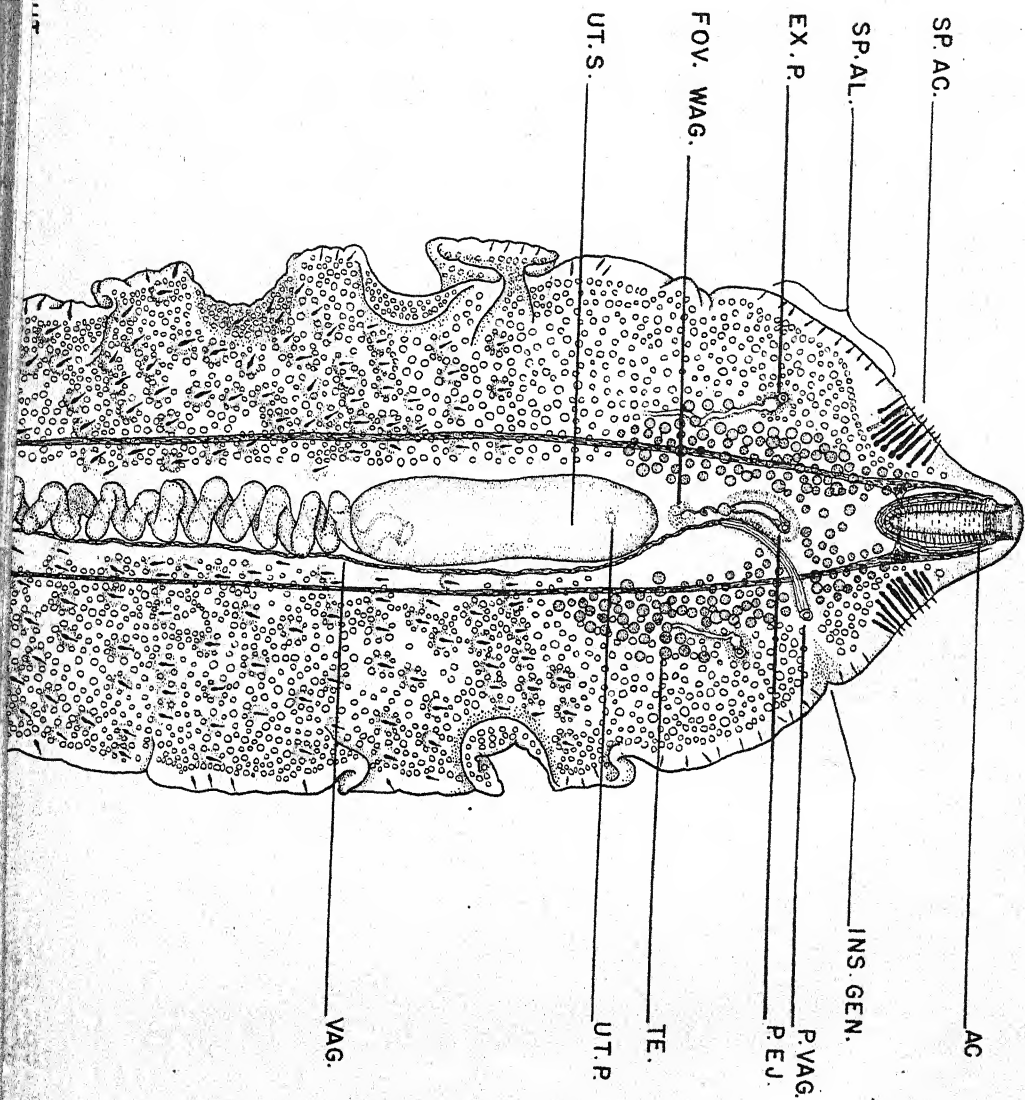
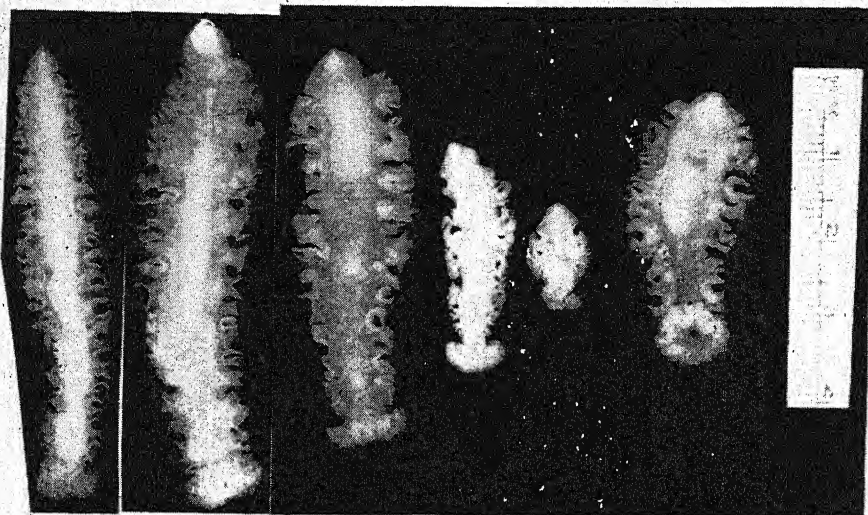
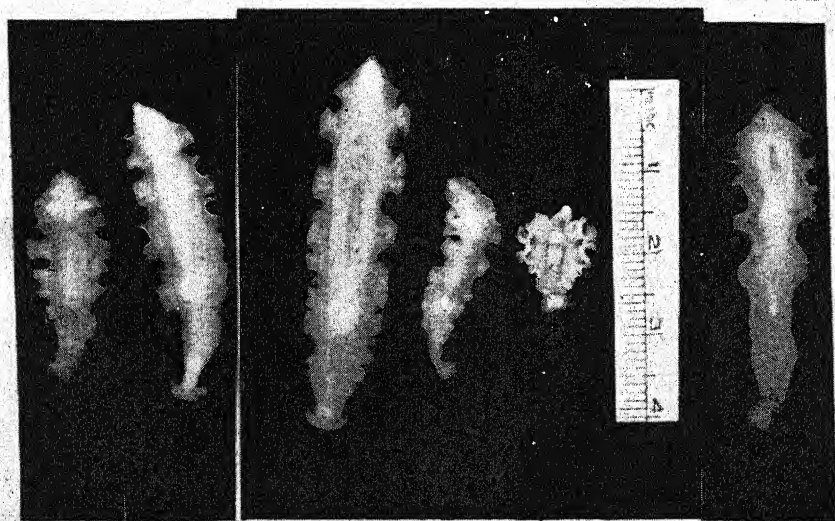


PLATE II  
Fig. 2. *Gyrocotyle urna*, dorsal aspect.  $\times 7.7$ .





3.



4.

## PLATE III

FIG. 3. Preserved specimens of *Gyrocotyle fimbriata*. Photographs by Dr. K. Bonham.

FIG. 4. Preserved specimens of *Gyrocotyle urna*. Photographs by Dr. K. Bonham. In the second and third specimens from left, the papillae in which the spines are embedded are readily visible in the posterior third of the dorsal surface.



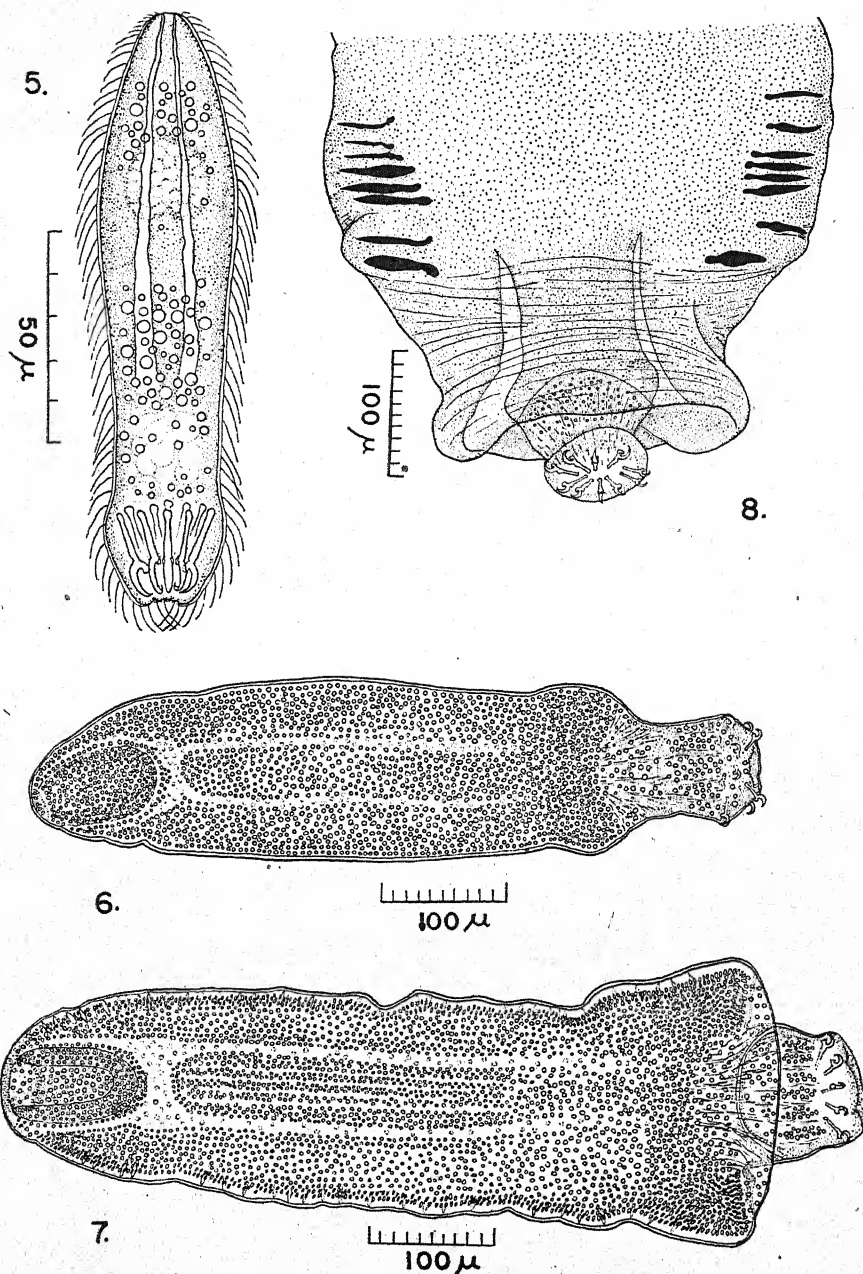


PLATE IV

FIG. 5. A decacanth larva of *Gyrocotyle fimbriata*. Drawing based on free-hand sketches of actively swimming larvae.  $\times 568$ .

FIG. 6. Early postlarval stage of *G. fimbriata*, from the intestine of the ratfish. The swelling anterior to the hook-bearing papilla presumably represents the Anlage of the rosette. Drawing of a stained whole mount.  $\times 168$ .

FIG. 7. Postlarval stage of *G. fimbriata*, from the intestine of a ratfish, showing early stage in the formation of the rosette. Drawing of a stained whole mount.  $\times 168$ .

FIG. 8. Posterior end of a postlarval *G. fimbriata* 1.5 mm in length. Note early stage in rosette formation, completely circular, which surrounds the hook-bearing papilla. Drawing of a stained whole mount.  $\times 168$ .



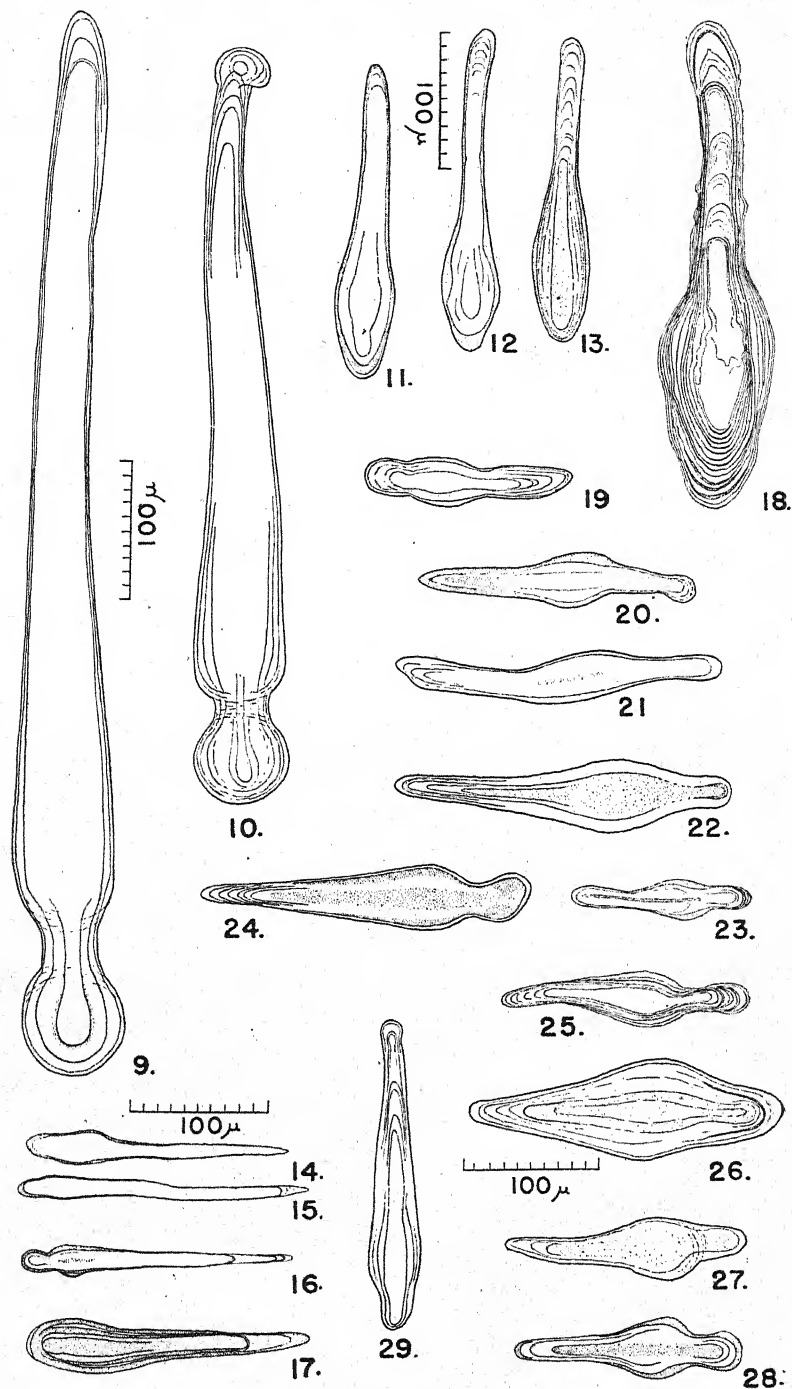


PLATE V

Figs. 10, 18 and 25 from specimens mounted in glycerine; the remainder from balsam mounts.  
All spines  $\times 183$ .

FIGS. 9-10. Acetabular spines of *G. urna magnispinosa*.

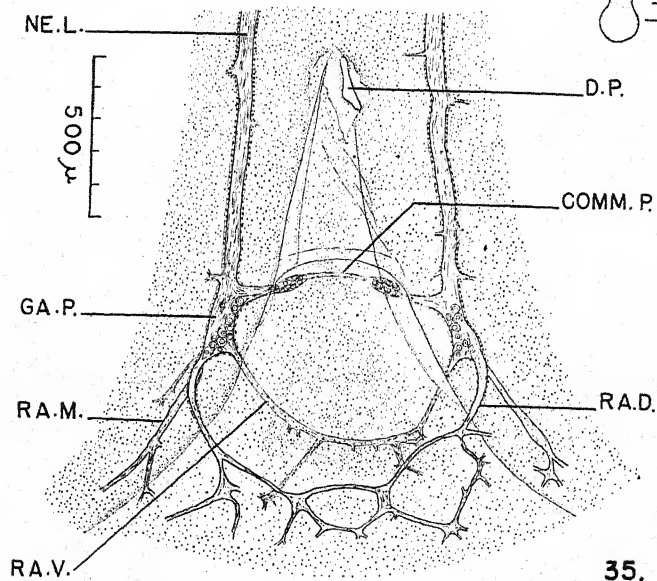
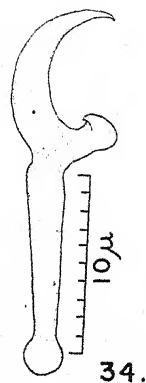
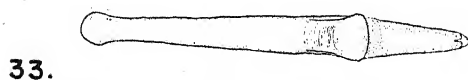
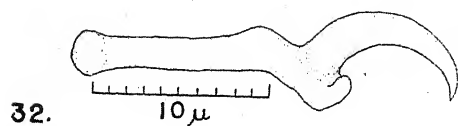
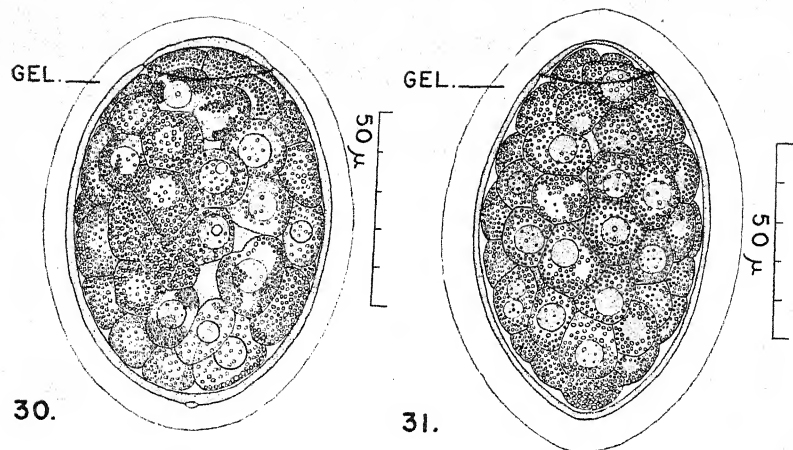
FIGS. 11-13. Posterodorsal spines of *G. urna magnispinosa*.

FIGS. 14-17. Acetabular spines of *G. urna parvispinosa*.

FIG. 18. A posterodorsal spine of *G. urna parvispinosa*.

FIGS. 19-23. Acetabular spines of *G. fimbriata*.

FIGS. 24-29. Posterodorsal spines of *G. fimbriata*.



# PLATE VI

FIG. 30. A spontaneously deposited egg of *G. fimbriata*.  $\times 515$ .

FIG. 31. A spontaneously deposited egg of *G. urna*.  $\times 515$ .

FIGS. 32-34. Hooks of the decacanth larva of *G. fimbriata*. Enlarged copies of camera lucida drawings.  $\times 2375$ .

FIG. 35. *Gyrocotyle urna*. Posterior end of the nervous system between the dorsal pore of the funnel and the rosette.  $\times 43$ .

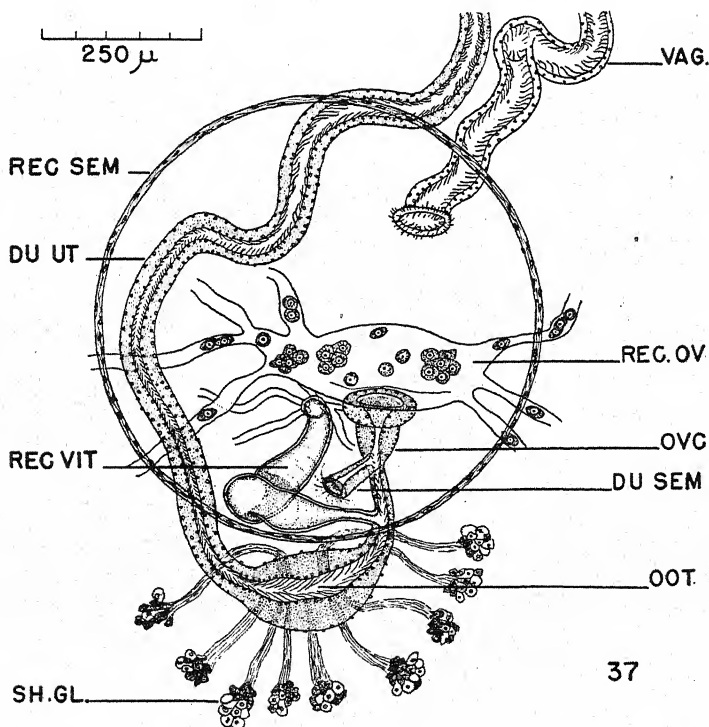
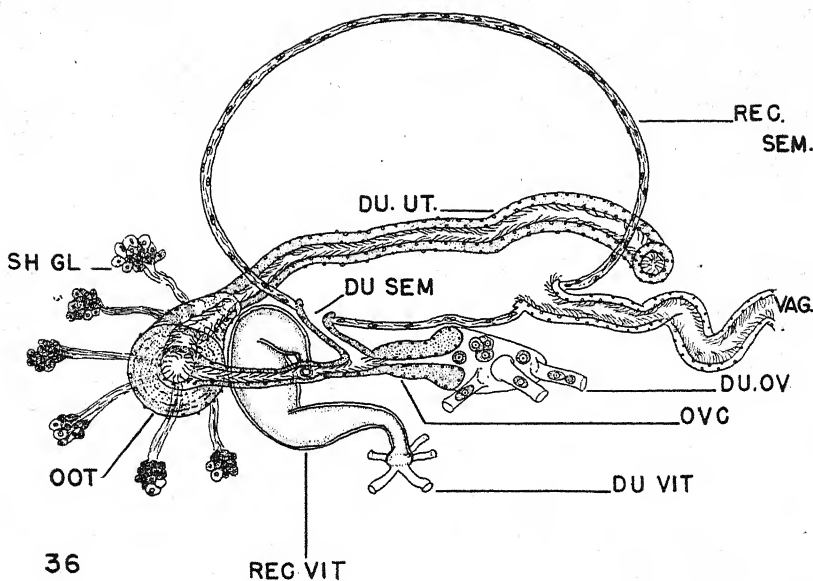


PLATE.VII

FIG. 36. Junction of the ducts of the female reproductive system and the beginning of the uterus in *G. fimbriata*, right lateral aspect. Reconstructed from serial sections.  $\times 85$ .

FIG. 37. The same, in dorsal view.  $\times 85$ .

In the interest of clarity, Figs. 36 and 37 are schematized as follows: (1) The bladderlike receptaculum seminis is depicted in outline, only. (2) The vitelline ducts and reservoir, normally crowded with yolk cells, are represented as empty. (3) The follicles of the shell gland are much more numerous than depicted in the drawings. (4) The seminal duct, normally dorsal to and nearly parallel to the oviduct, has had its proximal end shifted posteriorly.

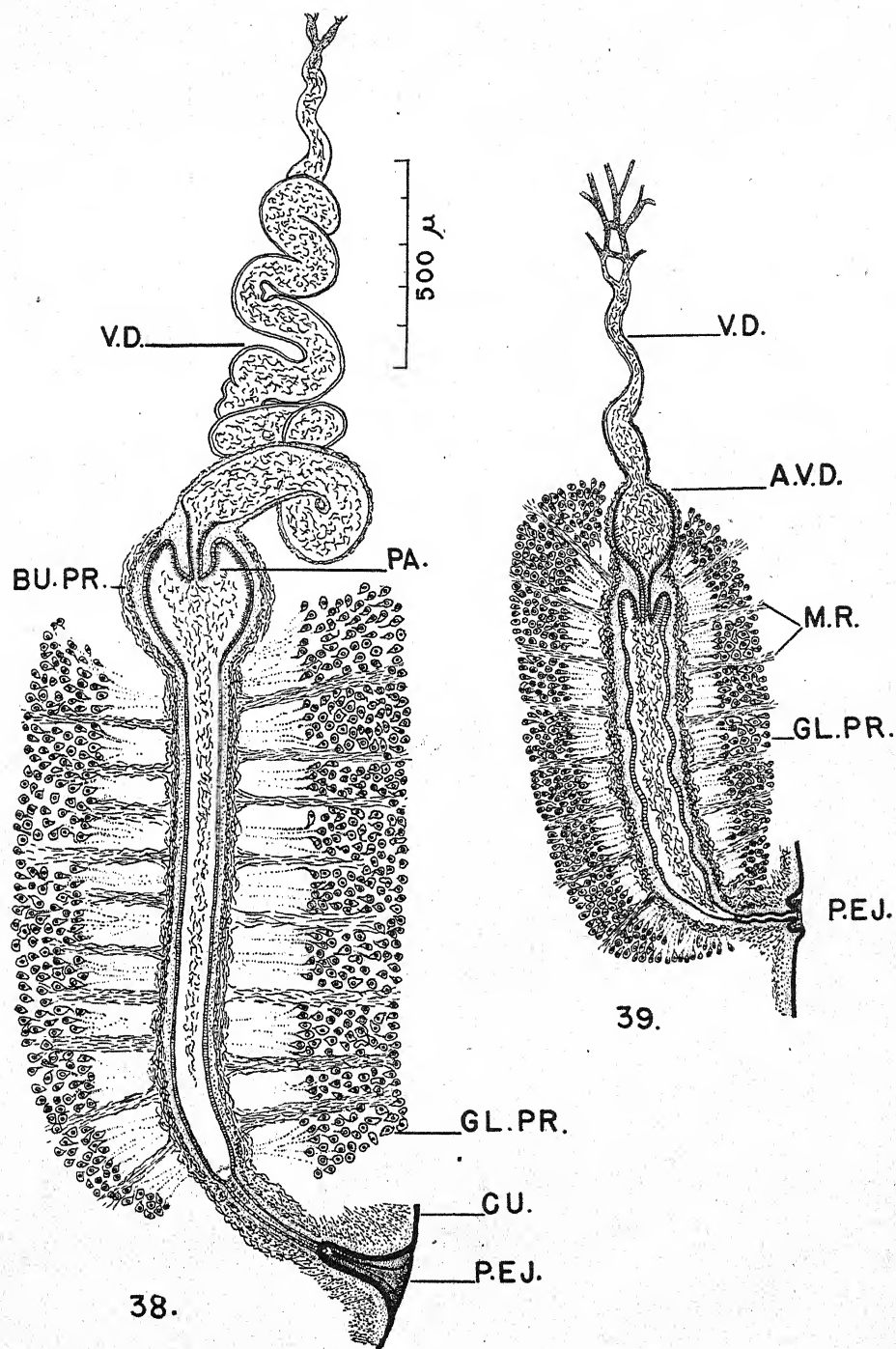


PLATE VIII

FIG. 38. Diagram of the ejaculatory duct and vas deferens in *G. fimbriata*, left lateral view.  $\times 56$ .

FIG. 39. The same, of *G. urna*.  $\times 56$ .

The magnification in Figs. 38 and 39 is not sufficient to show histological detail. The outline of Fig. 38 was traced with a camera lucida from a thick sagittal section; Fig. 39 was reconstructed from serial sections.



## BOOKS AND MONOGRAPHS RECEIVED

- BAKER, FRANK COLLINS (Curator, Museum of Natural History, Emeritus University of Illinois). **The Molluscan Family Planorbidae.** ix + 530 pp. 2 parts, 141 plates and illustrations, 9 pp. bibliography, 8 pp. index. Collation, Revision and Additions; 8 pp. a memorial to Frank Collins Baker (1867 to 1942) by Harley J. Van Cleave. Copyright 1945. The University of Illinois Press, Urbana. \$14.50.
- BENBROOK, EDWARD A. (V.M.D. Professor of Veterinary Pathology, Division of Veterinary Medicine, Iowa State College, Ames, Iowa). **List of Parasites of Domesticated Animals in North America.** i + 44 pp. No index. Copyright 1945. Burgess Publishing Co., Minneapolis, Minn.
- CARTER, CHARLES F. (B.S., M.D. Instructor in Microbiology and Pathology, Parkland Hospital School of Nursing, Dallas, Texas). **Microbiology and Pathology.** 3rd ed. 777 pp. 55 chapters, 25 color plates, 28 pp. glossary, 200 text-figures, 21 pp. index. Copyright 1944. The C. V. Mosby Co., St. Louis. \$3.50.
- CHANDLER, ASA C. (M.S., Ph.D. Professor of Biology, Rice Institute, Houston, Texas. Special Consultant Malaria Control in War Areas, U. S. Public Health Service). **Introduction to Parasitology.** 7th ed. iii + 716 pp. 26 chapters, 309 text-figures, 20 pp. index. Copyright 1944. John Wiley & Sons, New York. \$5.00.
- COFFIN, DAVID L. (V.M.D. School of Veterinary Medicine, University of Pennsylvania). **Manual of Veterinary Clinical Pathology.** v + 263 pp. 16 chapters, 3 color plates, 66 text-figures, 2 tables, 15 pp. index. Copyright 1945. Comstock Publishing Co., Inc., Ithaca, N. Y. \$4.00.
- COOLEY, R. A. (Senior Entomologist, Rocky Mountain Laboratory, Division Infectious Diseases, U. S. Public Health Service), AND GLEN M. KOHLS (Associate Entomologist, Rocky Mountain Laboratory, Hamilton, Mont.). **The Argasidae of North America, Central America and Cuba.** The American Midland Naturalist Monograph No. 1. 152 pp. 14 plates, 57 text-figures, 7 tables, 2 pp. index. Copyright 1944. The University Press, Notre Dame, Ind.
- CRAIG, CHARLES F. (M.D., M.A. (Hon.), F.A.C.S., F.A.C.P. Col. U. S. Army (Retired), D.S.M. Emeritus Professor of Tropical Medicine, Medical School, Tulane University of Louisiana, New Orleans). **The Etiology, Diagnosis, and Treatment of Amebiasis.** v + 332 pp. 12 chapters, 44 text-figures, 10 pp. references, 6 pp. author index, 6 pp. subject index. Copyright 1944. William & Wilkins Co., Baltimore. \$4.50.
- DESPAIGNE, DEMETRIO E. (Doctor, Director General Consejo Nacional de Tuberculosis, Cuba). **La Lucha Contra la Tuberculosis en Cuba.** 258 pp. 1 p. index. Copyright 1944. Publicaciones del Consejo Nacional de Tuberculosis, Habana, Cuba.
- FELSEN, JOSEPH (B.A., M.D. Director of Medical Research, Bronx Hospital, New York; Director of International and Pan-American Dysentery Registry). **Bacillary Dysentery Colitis and Enteritis.** v + 618 pp. 145 text-figures, 72 tables, 96 pp. bibliography, 10 pp. index. Copyright 1945. W. B. Saunders Co., Phila.
- HEADLEE, THOMAS J. (A.B., A.M., Ph.D. New Jersey Experiment Station, New Brunswick, N. J.). **The Mosquitoes of New Jersey and Their Control.** vii + 326 pp. 11 chapters. 16 plates, 87 text-figures, 14 tables, 2 pp. references. Copyright 1945. Rutgers University Press, New Brunswick. \$4.00.
- HOSKINS, MARGARET M. (A.B., Ph.D. Department of Anatomy, The Graduate School of Arts and Science and College of Dentistry, New York University), AND GERRIT BEVELANDER (A.M., Ph.D. Department of Anatomy, The Graduate School of Arts and Science and College of Dentistry, New York University). **Essentials of Histology.** 240 pp. 17 chapters, 2 color plates, 135 text illustrations, 7 tables, 14 pp. index. Copyright 1945. C. V. Mosby Co., St. Louis.
- MACKIE, THOMAS T. (Col. M.C., A.U.S. Executive Officer, Tropical and Military Medicine; Chief, Division of Parasitology, Army Medical School), GEORGE W. HUNTER III (Maj. Sn.C., A.U.S. Division of Parasitology, Army Medical School), AND C. BROOKE WORTH (Capt. M.C., A.U.S. Division of Parasitology, Army Medical School). **A Manual of Tropical Medicine** (Prepared under the auspices of the Division of Medical Sciences of the National Research Council). v + 727 pp. 11 sections, 66 tables, 284 text-figures, 287 illustrations (6 in color), 45 pp. index. Copyright 1945. W. B. Saunders Co., Phila.
- PARKER, JOHN B. (A.B., A.M., Ph.D. Professor Emeritus of Biology, The Catholic University of America), AND JOHN J. CLARKE (Ph.D. Assistant Professor of Biology (Retired), The Catholic University of America). **An Introduction to Animal Biology.** 2nd ed. 532 pp. 19 chapters, 172 text-figures, 7 pp. appendices, 14 pp. glossary, 17 pp. index. Copyright 1945. C. V. Mosby Co., St. Louis. \$3.75.

- ROGERS, LEONARD, SIR (K.C.S.I., C.I.E., L.L.D., M.D., B.S., F.R.C.P., F.R.S. Major-General, Indian Medical Service (Retired)), AND SIR JOHN W. D. MEGAW (K.C.I.E., B.A., M.B., Hon.D.Sc. (Queens University, Belfast). Major-General, Indian Medical Service (Retired)), *Tropical Medicine*. 5th ed. vii+518 pp. 7 sections, 28 chapters, 2 color plates, 87 text-figures, 3 tables, 12 pp. index. 1944. The Williams & Wilkins Co., Baltimore.
- STRONG, RICHARD P. (M.D., Sc.D., D.S.M., C.B. Professor of Tropical Medicine, Emeritus Harvard University. Consultant in Tropical Medicine to the Mass. General Hospital and the Boston City Hospital. Colonel M.C., U. S. Army; consultant to the Secretary of War and Director of Tropical Medicine, Army Medical School, Wash.). *Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases*. 7th ed. Vol. 1, vii+871+xl pp.; vol. 2, v+1747+xl pp. 8 sections, 54 chapters (and appendix 3 sections), color plates, 398 text-figures, tables, 40 pp. index. Copyright 1944. Blakiston Co., Philadelphia 5. \$21.00.

# INDEX FOR VOLUME 31, NOS. 1-6

<i>Acariscus</i> (Acarinida), two new species .....	401
ADDIS, C. J. Laboratory rearing and life cycle of <i>Phlebotomus</i> ( <i>Dampfomyia</i> ) <i>anthophorus</i> Addis (Diptera: Psychodidae) .....	319
ADDIS, C. J. <i>Phlebotomus</i> ( <i>Dampfomyia</i> ) <i>anthophorus</i> n. sp., and <i>Phlebotomus diabolicus</i> Hall from Texas (Diptera: Psychodidae) .....	119
ADDIS, C. J. (see Webster and Addis) .....	286
AMERICAN SOCIETY OF PARASITOLOGISTS:	
Minutes, 34th council meeting .....	152
Preliminary announcement of the 20th annual meeting .....	351
<i>Ancylostoma caninum</i> , response to larvae in human skin .....	366
<i>Anopheles</i> , new species from South Pacific .....	236, 241, 315
Anopheline mosquito of Solomon Islands and New Hebrides .....	241
<i>Ascaridia galli</i> (Nematoda), glycogen utilization by .....	406
BARTLETT, DAVID E. (see Hammond and Bartlett) .....	82
BELKIN, JOHN N. <i>Anopheles nataliae</i> , a new species from Guadalcanal .....	315
BELKIN, JOHN N., KENNETH L. KNIGHT AND LLOYD E. ROZEBOOM. Anopheline mosquitos of the Solomon Islands and New Hebrides .....	241
BELTRÁN, ENRIQUE. Conflicting views in regard to <i>Iodamoeba williamsi</i> .....	289
BISHOPP, F. C. AND H. L. TREMBLEY. Distribution and hosts of certain North American ticks .....	1
<i>Brachylecithum americanum</i> , a new liver fluke of birds .....	131
BRACKETT, STERLING AND CARRIE OLA HUGHES. Chilling as a means of retaining the viability of the sporozoites of <i>Plasmodium gallinaceum</i> .....	288
BRACKETT, STERLING (see Cort, Brackett, Olivier and Nolf) .....	61
CANTRELL, WILLIAM AND HELEN B. JORDAN. New mosquito hosts for <i>Plasmodium gallinaceum</i> .....	55
Cercarial penetration, influenced by metamorphosis of frog .....	205
Cestode cytology, studies in .....	213
CHANDLER, ASA C. <i>Trichuris</i> species from California rodents .....	284
Coccidiosis of chickens, effects of sulpha compounds on .....	352, 359
COLE, C. L. (see Hawkins and Cole) .....	113
<i>Corynosoma</i> (Acanthocephala), species in North American birds .....	332
CORT, W. W., STERLING BRACKETT, LOUIS OLIVIER AND L. O. NOLF. Influence of larval trematode infections in snails on their second intermediate host relations to the strigeid trematode, <i>Cotylurus flabelliformis</i> (Faust, 1917) .....	61
<i>Cotylurus flabelliformis</i> , host relations of .....	61
<i>Cricetus auratus</i> , host of <i>Hymenolepis nana</i> .....	151
CULLINAN, R. P. The larvae of <i>Eustrongylides ignotus</i> in <i>Fundulus heteroclitus</i> .....	109
DENTON, J. FRED. Studies on the life history of <i>Brachylecithum americanum</i> n. sp., a liver fluke of passerine birds .....	131
<i>Dermadena</i> , new trematode genus .....	411
<i>Dispharynx</i> (Nematoda), in birds .....	323
Ecology, of helminth parasites .....	142
<i>Eimeria tenella</i> , effects of sulpha compounds on .....	98, 359
<i>Endamoeba histolytica</i> , medium for encystation .....	155
<i>Endamoeba</i> , versus <i>Entamoeba</i> .....	177
<i>Entamoeba</i> , versus <i>Endamoeba</i> .....	177
<i>Eustrongylides ignotus</i> , larvae in <i>Fundulus heteroclitus</i> .....	109
<i>Eustrongylides</i> (Nematoda), respiratory metabolism of larvae .....	381
FARR, MARION M. (see Wehr and Farr) .....	359
FARR, MARION M. AND EVERETT E. WEHR. Sulfamerazine therapy in experimental cecal coccidiosis of chickens .....	353
FERNANDO, WILFRED. The storage of glycogen in the Temnocephaloidea .....	185
FRANKS, MYRON B. AND NORMAN R. STOLL. The isolation of microfilariae from blood for use as antigen .....	158

Gapeworms (Nematoda), in New York birds .....	394
GOBLE, FRANS C. AND H. L. KUTZ. The genus <i>Dispharynx</i> (Nematoda: Acuariidae) in galliform and passeriform birds .....	323
GOBLE, FRANS C. AND H. L. KUTZ. Notes on the gapeworms (Nematoda: Syngamidae) of galliform and passeriform birds in New York state .....	394
GRIFFITHS, JAMES T., JR. A scrub typhus (tsutsugamushi) outbreak in Dutch New Guinea	341
<i>Gyrocotyle</i> , morphology and relationships of .....	418
HAMMOND, DATUS M. AND DAVID E. BARTLETT. An instance of phagocytosis of <i>Trichomonas foetus</i> in bovine vaginal secretions .....	82
HAWKINS, PHILIP A. AND C. L. COLE. Studies of sheep parasites. V. Immunity to gastrointestinal nematodes .....	113
HERRICK, C. A. (see Ripson and Herrick) .....	98
HUGHES, CARRIE OLA (see Brackett and Hughes) .....	288
HUNTER, GEORGE W. III AND C. BROOKE WORTH. Variations in response to filariform larvae of <i>Ancylostoma caninum</i> in the skin of man .....	366
HUSSEY, KATHLEEN L. The miracidium of <i>Proterometra macrostoma</i> (Faust) Horsfall, 1933	269
<i>Hymenolepis nana</i> , in the golden hamster .....	151
<i>Hymenolepis nana</i> var. <i>fraterna</i> , effects of alcohol on resistance to .....	291
<i>Illiosentis</i> (Acanthocephala), a new species of .....	57
<i>Iodamoeba</i> , ingestion processes on .....	79
<i>Iodamoeba williamsi</i> , incidence of infection by .....	289
JELLISON, WILLIAM L. A new mite, <i>Laelaps aplodontiae</i> , from <i>Aplodontia</i> .....	373
JELLISON, WILLIAM L. Siphonaptera: the genus <i>Oropsylla</i> in North America .....	83
JONES, ARTHUR W. Studies in cestode cytology .....	213
JORDAN, HELEN B. (see Cantrell and Jordan) .....	55
KIRBY, HAROLD. <i>Entamoeba coli</i> versus <i>Endamoeba coli</i> .....	177
KIRBY, HAROLD. The structure of the common intestinal trichomonad of man .....	163
KNIGHT, KENNETH L. (see Belkin, Knight and Rozeboom) .....	241
KUTZ, H. L. (see Goble and Kutz) .....	323
KUTZ, H. L. (see Goble and Kutz) .....	394
<i>Laelaps apolodontiae</i> , new species of mite .....	373
LARSH, JOHN E. Effects of alcohol on natural resistance to the dwarf tapeworm in mice ...	291
LEIGH, W. HENRY AND HARLEY J. VAN CLEAVE. Metamorphosis of the frog host as a factor in cercarial penetration by <i>Glythelminis quieta</i> .....	205
Life history of <i>Brachylecithum americanum</i> .....	131
Life history of <i>Tamerlania bragai</i> .....	306
LYNCH, JAMES E. Redescription of the species of <i>Gyrocotyle</i> from the ratfish, <i>Hydrolagus coliei</i> (Lay and Bennet), with notes on the morphology and taxonomy of the genus ....	418
MALDONADO, JOSÉ F. The life cycle of <i>Tamerlania bragai</i> Santos, 1934 (Eucotylidae), a kidney fluke of domestic pigeons .....	306
MANter, HAROLD W. <i>Dermadena lactophrysi</i> n. gen., n. sp. (Trematoda: Lepocreadiidae) and consideration of the related genus <i>Pseudocreadium</i> .....	411
MATHESON, ROBERT. Descriptions of two new species, <i>Paratrichobius anduzei</i> and <i>Nycteribosca franclemonti</i> (Streblidae, Diptera, Pupipara) .....	191
MAUSS, EVELYN A. Pinworm infestation among children of rural communities .....	288
MELENEX, HENRY E. (see Zuckerman and Meleney) .....	155
Microfilariae, isolation of .....	158
Myiasis, by blowfly <i>Lucilia</i> sp. ....	151
<i>Neorcnifer crotali</i> , new trematode from rattlesnake .....	210
New genera (indicated*) and new species (volume 31, 1945):	
<i>Illiosentis cetratus</i> Van Cleave .....	57
<i>Phlebotomus anthophorus</i> Addis .....	119
<i>Polymorphus trochus</i> Van Cleave .....	128
<i>Brachylecithum americanum</i> Denton .....	131
<i>Paratrichobius anduzei</i> Matheson .....	191
<i>Nycteribosca franclemonti</i> Matheson .....	191



* <i>Paguritherium alatum</i> Reinhard .....	198
<i>Neoreniifer crotali</i> Self .....	210
<i>Anopheles kaliensis</i> Owen .....	236
<i>Anopheles solomonis</i> Belkin, Knight and Rozeboom .....	241
<i>Cercaria nyxetica</i> Seitner .....	272
<i>Cercaria neustica</i> Seitner .....	272
<i>Cercaria meringura</i> Seitner .....	272
<i>Cercaria nothrica</i> Seitner .....	272
<i>Cercaria tranoglandis</i> Seitner .....	272
<i>Trombicula frittsi</i> Wharton .....	282
<i>Trichuris citelli</i> Chandler .....	284
<i>Trichuris neotomae</i> Chandler .....	284
<i>Trichuris perognathi</i> Chandler .....	284
<i>Anopheles nataliae</i> Belkin .....	315
<i>Corynosoma anatarium</i> Van Cleave .....	332
<i>Laelaps aplodontiae</i> Jellison .....	337
<i>Acariscus pluvius</i> Wharton .....	401
<i>Acariscus anous</i> Wharton .....	401
* <i>Dermadena lactophrysi</i> Manter .....	411
* <i>Neoaxine</i> Price .....	Supplement abstract 60
NEWTON, WALTER L. AND IVAN PRATT. Experiments to determine whether infective larvae of <i>Wuchereria bancrofti</i> can migrate from the abdomen of the mosquito intermediate host .....	266
NOLF, L. O. (see Cort, Brackett, Olivier and Nolf) .....	61
<i>Nycteribosca</i> (Diptera), new species of .....	191
OLIVIER, LOUIS (see Cort, Brackett, Olivier and Nolf) .....	61
<i>Oropsylla</i> (Siphonaptera), distribution and hosts of North American species .....	83
OWEN, WILLIAM B. A new anopheline from the Solomon Islands with notes on its biology .....	236
<i>Paguritherium</i> (Crustacea), new entoniscian parasite of <i>Pagurus</i> .....	198
<i>Paratrachobius</i> (Diptera), new species of .....	191
<i>Phlebotomus anthophorus</i> (Diptera) rearing and life cycle .....	319
<i>Phlebotomus</i> , species from Texas .....	119
Pinworm (Nematoda), infection in rural children .....	288
<i>Plasmodium gallinaceum</i> , new mosquito hosts for .....	55
<i>Plasmodium gallinaceum</i> , viability of sporozoites .....	288
<i>Polymorphus</i> (Acanthocephala), new species from American coot .....	128
PRATT, IVAN (see Newton and Pratt) .....	266
<i>Proterometra macrostoma</i> (Trematoda), description of miracidium .....	269
<i>Pseudocercadium</i> (Trematoda), taxonomic relations of .....	411
RANKIN, JOHN S., JR. An ecological study of the helminth parasites of amphibians and reptiles of Western Massachusetts and vicinity .....	142
REID, W. MALCOLM. Comparison between <i>in vitro</i> and <i>in vivo</i> glycogen utilization in the fowl nematode <i>Ascaridia galli</i> .....	406
REINHARD, EDWARD G. <i>Paguritherium alatum</i> n. g., n. sp., an entoniscian parasite of <i>Pagurus longicarpus</i> .....	198
RICHARDS, CHARLES S. (see Scott, Richards and Seaman) .....	195
RIPSOM, C. A. AND C. A. HERRICK. Effects of various sulphur compounds on the protozoan parasite, <i>Eimeria tenella</i> .....	98
ROZEBOOM, LLOYD E. (see Belkin, Knight and Rozeboom) .....	241
SCHUCK, BETTY R. A new locality for <i>Trypanosoma cruzi</i> in Arizona .....	151
SCOTT, OLIVER W., CHARLES S. RICHARDS AND ELWOOD A. SEAMAN. Experimental infection of Southern California mosquitos with <i>Wuchereria bancrofti</i> .....	195
Scrub typhus, outbreak in New Guinea .....	341
SEAMAN, ELWOOD A. (see Scott, Richards and Seaman) .....	195
SEITNER, PHILIP G. Studies on five new species of Xiphidiocercariae of the <i>Virgula</i> type .....	272
SELF, J. TEAGUE. A new trematode, <i>Neoreniifer crotali</i> , from the rattlesnake .....	210
Sheep parasites, immunity to gastrointestinal nematodes .....	113
STABLER, ROBERT M. Ingestion processes on <i>Iodamoeba</i> (Protozoa) .....	79
STOLL, NORMAN R. (see Franks and Stoll) .....	158
STUNKARD, HORACE W. The morphology of <i>Tamerlania bragai</i> Dos Santos, 1934 .....	301

STUNKARD, HORACE W. The Syrian hamster, <i>Cricetus auratus</i> , host of <i>Hymenolepis nana</i> ..	151
Sulpha compounds, effect on <i>Eimeria tenella</i> .....	98
<i>Tameleria bragai</i> (Trematoda), life history of .....	306
<i>Tameleria bragai</i> (Trematoda), morphology of .....	301
Temnocephaloidea, storage of glycogen in .....	185
Ticks, distribution and hosts of .....	1
TREMBLEY, HELEN L. (see Bishopp and Tremblé) .....	1
<i>Trichomonas augusta</i> (Protozoa), cultivation of .....	375
<i>Trichomonas foetus</i> , phagocytosis of .....	82
Trichomonad of man, structure of .....	163
<i>Trichurus</i> (Nematoda), new species from California rodents .....	284
<i>Trombicula</i> (Acarina), new species of .....	282
<i>Trypanosoma cruzi</i> , in Arizona .....	151
VAN CLEAVE, HARLEY J. A new species of the acanthocephalan genus <i>Illioscutis</i> (Rhadinorhynchidae) .....	57
VAN CLEAVE, HARLEY J. A new species of the acanthocephalan genus <i>Polymorphus</i> from the American coot .....	128
VAN CLEAVE, HARLEY J. The acanthocephalan genus <i>Corynosoma</i> . I. The species found in water birds of North America .....	332
VAN CLEAVE, HARLEY J. (see Leigh and Van Cleave) .....	205
VON BRAND, THEODOR. Physiological observations upon a larval <i>Eustrongylides</i> . VIII. Influence of respiratory poisons upon the aerobic gaseous metabolism .....	381
WEBSTER, J. DAN. Intestinal myiasis with <i>Lucilia</i> .....	151
WEBSTER, J. DAN AND C. J. ADDIS. Helminths from the bob-white quail in Texas .....	286
WEHR, EVERETT E. (see Farr and Wehr) .....	353
WEHR, EVERETT E. AND MARION M. FARR. Effect of sulphaguanidine on the course of infection in chickens with <i>Eimeria tenella</i> .....	359
WENRICH, D. H. The cultivation of <i>Trichomonas augusta</i> (Protozoa) from frogs .....	375
WHARTON, G. W. <i>Trombicula fritti</i> n. sp. (Acarina: Trombiculidae) .....	282
WHARTON, G. W. Two new species of <i>Acariscus</i> : <i>A. pluvius</i> and <i>A. anous</i> (Acarinida: Trombiculidae) .....	401
WORTH, C. BROOKE (see Hunter and Worth) .....	366
<i>Wuchereria bancrofti</i> , migration of larvae in mosquito .....	266
<i>Wuchereria bancrofti</i> , in California mosquitos .....	195
Xiphidiocercariae, five new species of .....	272
ZUCKERMAN, LUCILE K. AND HENRY E. MELENEY. A fluid medium for the encystation of <i>Endamoeba histolytica</i> under reduced atmospheric pressure .....	155

# INDEX FOR DECEMBER SUPPLEMENT, 1945

(\* refers to abstract number rather than page)

ACKERT, J. E. (see Riedel and Ackert)	43*
ALLEN, REX W. Thermal death point of <i>Cysticercus bovis</i>	56*
AMERICAN SOCIETY OF PARASITOLOGISTS:	
<i>In memoriam</i>	29
Members elected 1944-1945	30
Officers	26
Program 20th annual meeting, St. Louis, Mo.	1
ANDERSON, DORCAS J. Life history of <i>Cercaria szidati</i>	50*
Author Index	6
AVERY, J. L. Periodicity of microfilariae in Philippines	55*
<i>Axine</i> (Trematoda), taxonomy of	60*
BARDES, CORRINE L. (see Wantland, Bardes and Levine)	57*
BEAVER, PAUL C. Immunity to <i>Necator americanus</i>	41*
BISCHOFF, ARTHUR I. (see Herman and Bischoff)	35*
BOZICEVICH, JOHN (see Hunter, Bozicevich and Warren)	23*
BRACKETT, STERLING AND EMANUEL WALETSKY. <i>Plasmodium gallinaceum</i> infection and pantothenic acid	15*
BRADFORD, MARY JANE AND C. A. HERRICK. Toxicity of <i>Eimeria tenella</i>	6*
BROOKE, M. M. Laboratory diagnosis of schistosomiasis	53*
BURGESS, ROBERT W. (see Young, Stubbs, Ellis, Burgess and Eyles)	25*
BYRD, ELON E. Epidemiology of filariasis in South Pacific	22*
CABLE, RAYMOND M. AND R. A. MCLEAN. Behavior of <i>Cercaria clausii</i>	51*
<i>Cercaria clausii</i> , swimming behavior of	51*
<i>Cercaria szidati</i> , life history of	50*
CHANDLER, ASA C. The making of a parasitologist	29*
COATNEY, G. ROBERT (see Cooper and Coatney)	10*
COATNEY, G. ROBERT (see Hershberger and Coatney)	11*
COATNEY, G. ROBERT AND W. CLARK COOPER. Quinine standardization in infections by <i>Plasmodium gallinaceum</i>	20*
Coccidiosis, prevented by sulfasuxidine	31*
Coccidiosis of rabbits, effects of sulpha compounds on	17*
COHEN, MINNA G. (see Hunter, Ingalls and Cohen)	54*
COOPER, W. CLARK (see Coatney and Cooper)	20*
COOPER, W. CLARK AND G. ROBERT COATNEY. <i>Plasmodium gallinaceum</i> infections in young chicks	10*
Cotton rat, hookworm of	42*
COULSTON, FREDERICK AND CLAY G. HUFF. Cryptozoites and metacryptozoites of <i>Plasmodium relictum</i>	8*
CRAM, ELOISE B. AND VIRGINIA S. FILES. Snail hosts of <i>Schistosoma mansoni</i>	27*
CROSS, JOY BARNES (see Scott and Cross)	33*
CROSS, JOY BARNES AND J. ALLEN SCOTT. Anatomy of <i>Litomosoides carinii</i>	37*
CULBERTSON, JAMES T. (see Rose and Culbertson)	39*
CULBERTSON, JAMES T. AND ELIZABETH PEARCE. Chemotherapy of filariasis in cotton rat	69*
<i>Cysticercus bovis</i> , thermal death point	56*
Cytochrome oxidase of pig ascarid	45*
Cytological techniques, application to helminth material	34*
<i>Diectophyme</i> (Nematoda), life history of	21*
DUSSEAU, ELIZABETH (see Porter, Laird and Dusseau)	12*
<i>Eimeria stieda</i> , relation of dosage to liver injury	7*
<i>Eimeria tenella</i> , effect of sulpha compounds on	16*
<i>Eimeria tenella</i> , toxicity of cecal cores from chickens	6*
ELLIS, JOHN M. (see Young, Stubbs, Ellis, Burgess and Eyles)	25*
<i>Endamoeba histolytica</i> , effect of whole egg on growth of	3*
<i>Endamoeba histolytica</i> , methods for detection of	1*

(\* refers to abstract number rather than page)

<i>Endamoeba histolytica</i> , studies on media for .....	18*
ENGLEY, FRANK B. (see Stabler and Engley) .....	4*
EYLES, DON E. (see Young, Stubbs, Ellis, Burgess and Eyles) .....	25*
FALLIS, A. MURRAY <i>Plasmodium circumflexum</i> in ruffed grouse .....	9*
<i>Fasciola hepatica</i> , ecology and control .....	49*
FELSENFIELD, OSCAR Methods for detection of <i>Endamoeba histolytica</i> .....	1*
Filariasis, epidemiology of .....	22*
Filariasis, skin tests for .....	23*
Filariasis, treatment with trivalent arsenicals .....	38*
Filariasis, treatment of human cases .....	39*
Filariasis of cotton rat, chemotherapy of .....	69*
FILES, VIRGINIA S. (see Cram and Files) .....	27*
Fowl ascarid, resistance to .....	43*
GOLDFISCHER, RHODA (see Wilmoth and Goldfisher) .....	58*
GOODCHILD, CHAUNCEY G. Life history of <i>Gorgoderia amplicava</i> .....	61*
<i>Gorgoderia</i> (Trematoda), life history of .....	61*
HARWOOD, PAUL D. Dosage of phenothiazine .....	44*
HAUSCHKA, THEODORE S., N. DU BOSE MAXWELL AND ELEANOR M. JOHNSON. Hexenolactone, inhibitor of <i>in vitro</i> growth of <i>Trypanosoma cruzi</i> .....	19*
HAWKINS, PHILIP A. Weather and nematode parasites of sheep .....	40*
HERMAN, CARLTON M. AND ARTHUR I. BISCHOFF. Distribution of <i>Onchocerca cervipedis</i> ..	35*
HERRICK, C. A. (see Bradford and Herrick) .....	6*
HERRICK, C. A. AND MARIE THEDE. Cytochrome oxidase of pig ascarid .....	45*
HERSHBERGER, LLOYD R. AND G. ROBERT COATNEY. Pathology of <i>Plasmodium gallinaceum</i> ..	11*
HUFF, CLAY G. (see Coulston and Huff) .....	8*
HUGHES, CARRIE OLA (see Waletsky and Hughes) .....	16*
HUNTER, GEORGE W. III, JOHN BOZICEVICH AND VIRGINIA G. WARREN. Skin tests for filariasis .....	23*
HUNTER, GEORGE W. III, JAMES W. INGALLS AND MINNA G. COHEN. Diagnosis of <i>Schistosoma japonicum</i> .....	54*
<i>Hymenolepis</i> (Cestoda), resistance to .....	47*
INGALLS, JAMES W. (see Hunter, Ingalls and Cohen) .....	54*
JANKIEWICZ, HARRY A. <i>Eimeria stieda</i> and liver coccidiosis .....	7*
JANKIEWICZ, HARRY A. Liver coccidiosis prevented by sulfasuxidine .....	31*
JOHNSON, ELEANOR M. (see Hauschka, Maxwell and Johnson) .....	19*
JOHNSON, ELEANOR M. (see Rees, Reardon, Johnson and Mayfield) .....	3*
JONES, ARTHUR W. AND HELEN L. WARD. Cytology of cestodes and other helminths .....	34*
LAIRD, RAYMOND L. (see Porter, Laird and Dusseau) .....	12*
LAIRD, RAYMOND L. AND RICHARD J. PORTER. <i>Plasmodium cynomolgi</i> infections .....	13*
LARSH, JOHN E., JR. Resistance to <i>Hymenolepis</i> .....	47*
LEVINE, ROBERT S. (see Wantland, Bardes and Levine) .....	57*
LEVITAS, NORA (see Wilmoth and Levitas) .....	59*
Life history of <i>Cercaria szidati</i> .....	50*
Life history of <i>Diectophyme renale</i> .....	21*
Life history of <i>Gorgoderia amplicava</i> .....	61*
<i>Litomosoides carinii</i> , growth rate of .....	36*
<i>Litomosoides</i> (Nematoda), morphology .....	37*
<i>Litomosoides</i> (Nematoda), and tumor formation .....	33*
LUND, EVERETT E. Effect of phthalylsulfathiazole on coccidiosis of rabbits .....	17*
Malarial sporozoites, <i>in vitro</i> studies on .....	12*
MALDONADO, JOSÉ F. (see Morales and Maldonado) .....	67*
MAREN, THOMAS H. (see Otto and Maren) .....	38*
MAXWELL, N. DU BOSE (see Hauschka, Maxwell and Johnson) .....	19*
MAYFIELD, M. FRANCES (see Rees, Reardon, Johnson and Mayfield) .....	3*
MAYHEW, ROY L. Effects of nodular worm .....	46*
MCLEAN, R. A. (see Cable and McLean) .....	51*



(\* refers to abstract number rather than page)

Microfilariae, periodicity of .....	55*
MORALES, F. HERNANDEZ AND JOSÉ M. MALDONADO. Diagnosis of schistosomiasis <i>mansoni</i> ..	67*
MORGAN, BANNER BILL. Studies on <i>Trichomonas foetus</i> .....	5*
<i>Necator</i> (Nematoda), immunity to .....	41*
Nematode parasites of sheep .....	40*
NIGRELLI, ROSS F. Parasites of bassalian fishes .....	62*
Nodular worm, effects in calves .....	46*
NOLF, L. O. Cercariae and metacercariae in Wisconsin Lake .....	48*
OFFUTT, EDWARD P., JR. AND IRA R. TELFORD. <i>Sarcocystis</i> in the monkey .....	30*
OLSEN, O. WILFORD. Metacercariae of <i>Fasciola hepatica</i> .....	49*
<i>Onchocerca cervipedis</i> , distribution of .....	35*
<i>Opelcoeloides</i> , new species of .....	32*
<i>Ostiolum</i> (Trematoda), glycogen distribution in .....	58*
<i>Ostiolum</i> (Trematoda), respiration of .....	59*
OTTO, GILBERT F. AND THOMAS H. MAREN. Canine filariasis and trivalent arsenicals .....	38*
<i>Paramoecium bursaria</i> , parasitized by bacteria .....	68*
Parasites of bassalian fishes .....	62*
Parasites, nematodes of Bufonidae .....	64*, 65*, 66*
Parasites of quail in Mississippi .....	63*
PEARCE, ELIZABETH (see Culbertson and Pearce) .....	69*
Phenothiazine, effects in fowls .....	44*
<i>Plasmodium circumflexum</i> , in ruffed grouse .....	9*
<i>Plasmodium cynomolgi</i> , course of infection by .....	13*
<i>Plasmodium elongatum</i> , infection in Pekin ducks .....	14*
<i>Plasmodium gallinaceum</i> , effect of panthothenic acid on infection by .....	15*
<i>Plasmodium gallinaceum</i> , infections in young chicks .....	10*
<i>Plasmodium gallinaceum</i> , pathology in young chicks .....	11*
<i>Plasmodium gallinaceum</i> , quinine standardization in infections .....	20*
<i>Plasmodium relictum</i> , unpigmented stages in canaries and pigeons .....	8*
<i>Plasmodium vivax</i> , foreign strains in American mosquitos .....	25*
PORTER, RICHARD J. (see Laird and Porter) .....	13*
PORTER, RICHARD J., RAYMOND L. LAIRD AND ELIZABETH DUSSEAU. <i>In vitro</i> studies on malarial sporozoites .....	12*
PRICE, EMMETT W. The genus <i>Axine</i> .....	60*
Protozoa, staining of, in human feces .....	2*
REARDON, LUCY V. (see Rees, Reardon, Johnson and Mayfield) .....	3*
REARDON, LUCY V. (see von Brand, Rees, Reardon and Simpson) .....	18*
REES, CHARLES W. (see von Brand, Rees, Reardon and Simpson) .....	18*
REES, CHARLES W., LUCY V. REARDON, ELEANOR M. JOHNSON AND M. FRANCES MAYFIELD. Influence of whole egg on growth of <i>Endamoeba histolytica</i> .....	3*
RIEDEL, B. B. AND J. E. ACKERT. Resistance to fowl ascarid .....	43*
ROSE, HARRY M. AND JAMES T. CULBERTSON. Treatment of human filariasis .....	39*
<i>Sarcocystis</i> , in the monkey .....	30*
<i>Sarcocystis</i> , is a mold belonging to genus <i>Aspergillus</i> .....	24*
<i>Schistosoma japonicum</i> , biology of infection .....	28*
<i>Schistosoma japonicum</i> , methods of diagnosis .....	54*
<i>Schistosoma mansoni</i> , biology of infection .....	28*
<i>Schistosoma mansoni</i> , snail hosts of .....	27*
Schistosomiasis, diagnosis of .....	67*
Schistosomiasis, general account .....	52*
Schistosomiasis, laboratory diagnosis of .....	53*
SCOTT, J. ALLEN. Growth rate of <i>Litomosoides carinii</i> .....	36*
SCOTT, J. ALLEN (see Cross and Scott) .....	37*
SCOTT, J. ALLEN AND JOY BARNES CROSS. <i>Litomosoides carinii</i> and tumor formation .....	33*
Scrub typhus, vectors of .....	26*
SIMPSON, WILLIAM F. (see von Brand, Rees, Reardon and Simpson) .....	18*

(\* refers to abstract number rather than page)

SPINDLER, L. A. AND HARRY E. ZIMMERMAN, JR. Biological status of <i>Sarcocystis</i> .....	24*
STABLER, ROBERT M. AND FRANK B. ENGLE. Pathogenicity of <i>Trichomonas gallinae</i> to pigeons .....	4*
STUBBS, TRAWICK H. (see Young, Stubbs, Ellis, Burgess and Eyles) .....	25*
TELFORD, IRA R. (see Offutt and Telford) .....	30*
THEDE, MARIE (see Herrick and Thede) .....	45*
THOMAS, LYELL J. Hookworm of the cotton rat .....	42*
Trichiniasis, encapsulation of larvae .....	57*
<i>Trichomonas foetus</i> , inoculation and immunization .....	5*
<i>Trichomonas gallinae</i> , pathogenicity for pigeons .....	4*
<i>Trypanosoma cruzi</i> , inhibition of growth <i>in vitro</i> .....	19*
VON BRAND, THEODOR, CHARLES W. REES, LUCY V. REARDON AND WILLIAM F. SIMPSON. Egg white medium for <i>Endamoeba histolytica</i> .....	18*
VON WICKLEN, JANE HOGAN New species of <i>Opecocloides</i> .....	32*
WALETSKY, EMANUEL (see Brackett and Waletsky) .....	15*
WALETSKY, EMANUEL AND CARRIE OLA HUGHES. Effects of sulpha compounds on infections by <i>Eimeria tenella</i> .....	16*
WALTON, A. C. Nematode parasites of Bufonidae, I, II and III .....	64*, 65*, 66*
WANTLAND, WAYNE W., CORRINE L. BARDES AND ROBERT S. LEVINE. Encapsulation in trichiniasis .....	57*
WARD, HELEN L. (see Jones and Ward) .....	34*
WARD, J. W. Parasites of quail in Mississippi .....	63*
WARREN, VIRGINIA G. (see Hunter, Bozicevich and Warren) .....	23*
WEINSTEIN, PAUL P. Schistosomiasis .....	52*
WHARTON, G. W. Vectors of scrub typhus .....	26*
WICHTERMAN, RALPH Schizomycetes parasitic in <i>Paramoecium bursaria</i> .....	68*
WILMOTH, JAMES H. AND RHODA GOLDFISCHER. Glycogen distribution in frog lung fluke ..	58*
WILMOTH, JAMES H. AND NORA LEVITAS. Respiration in frog lung fluke .....	59*
WOLFSON, FRUMA <i>Plasmodium elongatum</i> in Pekin ducks .....	14*
WOODHEAD, ARTHUR E. Life history of <i>Diectophyme renale</i> .....	21*
WRIGHT, WILLARD H. Biology of <i>Schistosoma</i> infections .....	28*
YOUNG, MARTIN D., TRAWICK H. STUBBS, JOHN M. ELLIS, ROBERT W. BURGESS AND DON E. EYLES. Foreign malarias in anopheline mosquitos of southern United States .....	25*
YOUNG, VIOLA MAY Staining of protozoa in stool specimens .....	2*
ZIMMERMAN, HARRY E., JR. (see Spindler and Zimmerman) .....	24*